FORMULATION AND *IN-VITRO* EVALUATION OF RALOXIFENE HYDROCHLORIDE LOADED MIXED PLURONIC L121/F127 POLYMERIC MICELLES

A Dissertation submitted to
THE TAMIL NADU Dr. M.G.R MEDICAL UNIVERSITY
CHENNAI – 600 032

In partial fulfillment of the requirements for the award of degree of

MASTER OF PHARMACY
IN
PHARMACEUTICS

Submitted by
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COLLEGE OF PHARMACY
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MAY – 2017

DEPARTMENT OF PHARMACEUTICS
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CHENNAI-600 003
TAMILNADU

DATE:

CERTIFICATE

This is to certify that the dissertation entitled **"FORMULATION AND *IN-VITRO* EVALUATION OF RALOXIFENE HYDROCHLORIDE LOADED MIXED PLURONIC L121/F127 POLYMERIC MICELLES"** submitted by **Mrs. JAMEELA JASMINE H.** with **Register No.261511252**for The Tamil Nadu Dr. M.G.R. Medical University is evaluated.

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Place: Chennai – 03

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(Dr. A. JERAD SURESH, M.Pharm., Ph.D., M.B.A)

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Date:

[Prof. K.ELANGO, M.Pharm.,(Ph.D.)]

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ABBREVIATIONS AND SYMBOLS

DDS	-	Drug Delivery Systems
RES	-	Reticulo Endothelial System
CMC	-	Critical Micelle Concentration
MPS	-	Mononuclear Phagocytic System
PEG	-	Poly Ethylene Glycol
PEO	-	Poly Ethylene Oxide
PPO	-	Poly Propylene Oxide
PEO-PPO-PEO	-	Poly(ethylene oxide)-Poly(propylene oxide)-Poly(ethylene oxide)
KDa	-	Kilo Daltons
PMs	-	Polymeric Micelles
PDI	-	Poly Dispersity Index
PBS	-	Phosphate Buffered Saline
TEM	-	Transmission Electron Microscopy
ERα	-	Estrogen Receptor α
ERβ	-	Estrogen Receptor β
RXH	-	Raloxifene Hydrochloride
BMD	-	Bone Mineral Density
SERMs	-	Selective Estrogen Receptor Modulators
RANKL	-	Receptor Activation of Nuclear Factor-β kb Ligand
OPG	-	Osteoprotegerin
FTIR	-	Fourier Transform Infra Red
UV-Vis Spectroscopy	-	Ultra violet Visible Spectroscopy
OBs	-	Osteoblasts
OCs	-	Osteoclasts
BMUs	-	Basic Multicellular Units
IL	-	Interleukin

TNF	-	Tumor Necrosis Factor
C-Fms	-	Colony Stimulating Factor receptor 1
ERT	-	Estrogen Replacement Therapy
TGF	-	Tumor Growth Factor
LDL	-	Low Density Lipoprotein
MORE	-	Multiple Outcomes Of Raloxifene Evaluation
HRT	-	Hormone Replacement Therapy
PTH	-	Para Thyroid Hormone
IU	-	International Unit
CAS Number	-	Chemical Abstracts Service Number
BPC	-	British Pharmacopoeial Commission
AHFS	-	American Hospital Formulary Service
GI	-	Gastro Intestine
LH	-	Leutinizing Hormone
FSH	-	Follicle Stimulating Hormone
LPS	-	Lipopolysaccharide
HLB	-	Hydrophilic Lipophilic Balance
EE%	-	Percentage Entrapment Efficiency
ASHP	-	American Society of Health System
MOHFW	-	Ministry Of Health and Family Welfare
mV	-	milliVolt
rpm	-	revolution per minute
g	-	gram
mg	-	Milligram
ml	-	Milliliter
µg	-	Microgram

%	-	Percentage
M.W	-	Molecular Weight
º	-	Degree
nm	-	nanometer
SD	-	Standard Deviation

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DRUG DELIVERY SYSTEMS

The method by which a drug is delivered can have a significant effect on its efficacy. Some drugs have an optimum concentration range within which maximum therapeutic effect is achieved and concentrations above or below this range can be toxic or produce no therapeutic benefit at all. On the other hand, the very slow progress in the efficacy of the treatment of severe diseases has suggested a growing need for a multidisciplinary approach for the delivery of therapeutics to targets in tissues (Bhagwat & Vaidhya, 2013).

New ideas on controlling the pharmacokinetics, pharmacodynamics, non-specific toxicity, immunogenicity, biorecognition and efficacy of drugs, a strategy called drug delivery systems (DDS) were generated, which are based on interdisciplinary approaches that combine polymer science, pharmaceutics, bioconjugate chemistry, and molecular biology. To minimize drug degradation and loss,to increase drug bioavailability and fraction of the drug accumulated in the required zone and to prevent harmful side effects various drug delivery and drug targeting system are currently under development (Bhagwat & Vaidhya, 2013).

Various Drug Delivery Systems:

Carrier based Drug Delivery System:

-Liposomes

-Nanoparticles

-Microspheres

-Monoclonal antibodies

-Niosomes

-Resealed erythrocytes as drug carriers

Transdermal Drug Delivery Systems:

-Sonophoresis

-Osmotic pump

-Microencapsulation

DRUG DELIVERY CARRIERS:

Colloidal drug carrier systems such as micellar solutions, vesicle and liquid crystal as well as nanoparticle dispersions consisting of small particles of 10-400 nm diameter show great promise as drug delivery system . The goal of developing these formulations is to obtain systems with optimized drug oading and release properties ,low toxicity and long shelf life (Aruna Rastogi, n.d.).

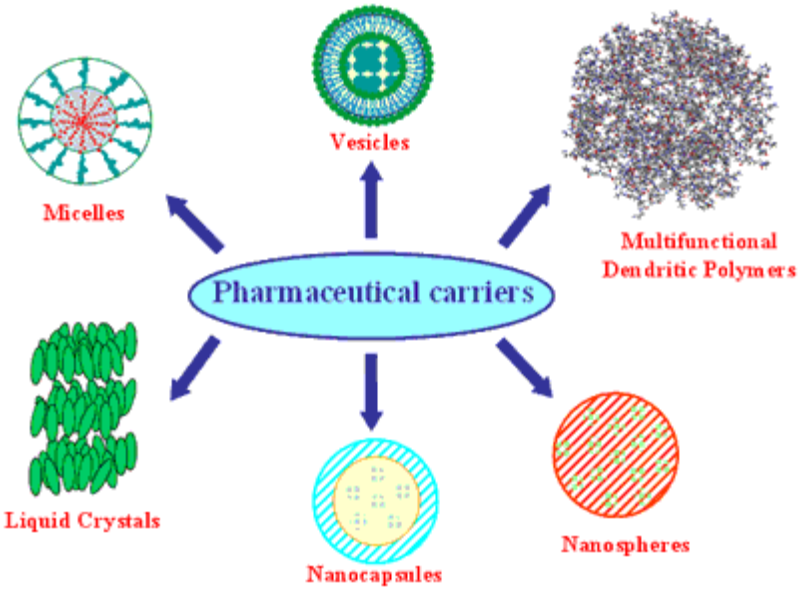


Figure 1. Different pharmaceutical carriers

MICELLAR SYSTEMS

Micelles formed by self-assembly of amphiphilic block copolymers (5-50 nm) in aqueous solutions are of great interest in drug delivery applications. The drugs can be physically entrapped in the core of block copolymer micelles and transported at concentrations that can exceed their intrinsic water-solubility. Moreover the hydrophilic blocks can form hydrogen bonds with aqueous surroundings and form a tight shell around the micellar core. As a result the contents of the hydrophobic core are effectively protected against hydrolysis and enzymatic degradation. In addition the corona may prevent recognition by the reticuloendothelial system (RES) and therefore preliminary elimination of the micelles from the bloodstream (Bhagwat & Vaidhya, 2013).

A final attractive feature of amphiphilic block copolymers is that their chemical composition, total molecular weight and block length ratios can be easily changed, which allows control of the size and morphology of the micelles (Bhagwat & Vaidhya, 2013).

POLYMERIC MICELLES

Copolymers with surfactant characteristics can also be used to formulate micelles. Micelles formed from copolymers tend to have a relatively narrow size distribution compared to standard surfactant micelles. Generally, they also have a lower critical micelle concentration (CMC) and are more stable. Due to their low CMCs, polymeric micelles are relatively insensitive to dilution, thus preventing their rapid dissociation and enhancing their circulation time compared to surfactant micelles. Polymeric micelles are built from copolymers with hydrophobic components comprising poly(propylene oxide), poly (D,L-lactic acid), poly(ε-caprolactone), poly(L-aspartate) and poloxamers. For the hydrophilic component, which forms the outer hydrophilic shell of the micelle, PEG is commonly used.The use of PEG as the hydrophilic component supports the formation of micelles. The hydrated PEG surface created on the micelles enhances their plasma half-life by promoting steric hindrance and blocking enzymes and antibodies reaching the drug, thereby offering protection to the drug and blocking interactions with the mononuclear phagocytic system (MPS).(As in case of PEO, the highly swollen and flexible shell may play a crucial role in diminishing the recognition of RES cells towards the polymeric micelles –(55)). As the micelles are sufficiently large (>50 kDa) to avoid renal excretion yet small enough (<200 nm) to avoid clearance by the liver and spleen, they are able to promote the specific accumulation of the micelles at tumour sites and sites of inflammation due to passive targeting (Yvonne Perrie, 2013)

As for dendrimers, the outer surface of the polymeric micelles can be further functionalized with targeting groups (such as folate, sugar residues or proteins) to promote their application in drug delivery and targeting. The attachment of monoclonal antibodies to reactive groups incorporated in the hydrophilic coating of polymeric micelles has also been investigated and shown to promote specific interaction of the micelles with antigens corresponding to the antibodies. These micelles are often referred to as immunomicelles (Yvonne Perrie, 2013)

CHEMICAL NATURE (Pavan Kumar Reddy *et al.,* 2015)

The research work on polymeric micelles has mainly concentrated on copolymers having an A-B diblock structure with A, the hydrophilic (shell) and B, the hydrophobic polymers (core) respectively. Polymeric micelles formation includes multiblock copolymers such as poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) (PEO-PPO-PEO) (A-B-A) which self-organizes to form the micelles. During the micelle formation drugs can be incorporated to form drug carrier systems.

The hydrophobic core which generally consists of a biodegradable polymer such as poly(propylene oxide) (PPO), poly(*b*-benzyl-1-aspartate) (PBLA), poly(di-lactic acid) (PDLLA) or poly (e-caprolactone) acts as a reservoir for an hydrophobic drug and protects it from contacting with aqueous environment.

The core may also be a water soluble polymer (e.g., poly(aspartic acid)) which is rendered hydrophobic by the chemical conjugation of a hydrophobic drug or through association between two oppositely charged polyions (polyion complex micelles).

The poly(ethylene oxide) (PEO) or polyethylene glycol (PEG) is the most commonly used material to form hydrophilic shell. The shell is responsible for micelle stabilization and interactions with cell membranes and plasmatic proteins. The biodistribution of polymeric system is usually dictated by nature of these hydrophilic shell.

BENEFITS OF USING PLURONICS (Poly(ethylene oxide)-poly(propylene oxide) block copolymers): (Diego Chiapetta & Alejandro Sosnik, 2007)

Micelles formed using pluronic copolymers have following advantages.

- Polymeric micelles are kinetically stable so they dissociate slowly, even at concentrations below the CMC, extending circulation times in blood.
- Micelles with blocks made of poly(ethylene oxide) are sterically stabilized(stealth) and undergo less opsinization and uptake by the macrophages of the reticuloendothelialsystem (RES), allowing the micelles to circulate longer in blood.
- Even though PEO-PPO-PEO materials are non-degradable, molecules with a molecular weight in the 10-15kDa range are usually filtered by the kidney and cleared in the urine.

MECHANISM OF MICELLE FORMATION (Pavan Kumar Reddy *et al.*, 2015)

There are two forces which helps in formation of micelles

-An attractive force which leads to the association of molecules and

-A repulsive force which prevents the infinite growth of the micelles to a distinct macroscopic phase.

The process of micellization of amphiphilic copolymers is similar to the process for surfactants. At low concentrations the polymers exist as single chains and as the concentration increases to its critical value called as the critical micelle concentration (CMC) the polymer chains start to associate and form micelles. In this system, when water is used as a solvent, the hydrophobic core part of the copolymer avoid contact with aqueous environment while forming micellar structure

BIODISTRIBUTION AND STABILITY OF MICELLE STRUCTURE

The threshold concentration for assembly of polymeric micelles is critical micellar concentration. Polymeric micelles may not necessarily dissociate immediately after extreme dilution because they have a remarkably low CMC ($10^{-6} – 10^{-7}$) which is 1000 folds lower than that of surfactant micelles (Nobuhiro Nishiyama & Kazunori Kataoka, 2006). Polymeric micelles

behave as large particle that evade the renal excretion during delivery to their site of action, while they eventually disassemble into small single polymer chains that can be excreted from the bloodstream at kidney (Masayuki Yokoyama, 2014).

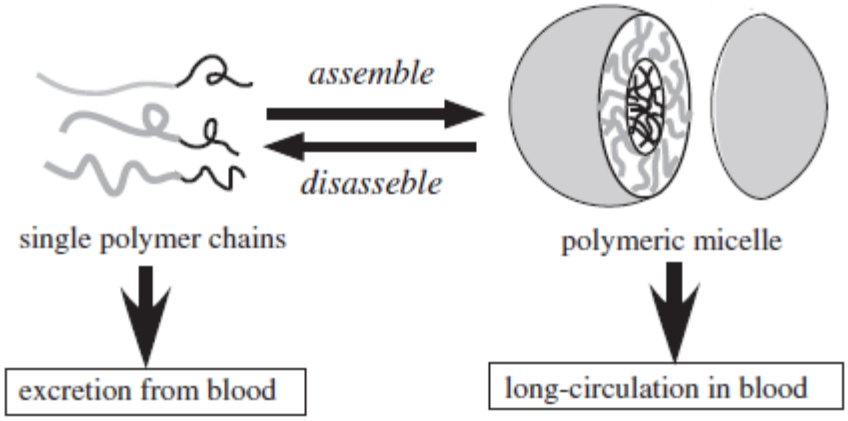


Figure 2. Polymeric micelles as drug carriers

The disassembling rate is an important factor for a good balance between the targeting efficiency and the low toxicity and it is also important for drug release rate because drugs are expected to be released instantaneously upon the disassembly of micelle structures (Masayuki Yokoyama, 2014).

PREPARATION OF DRUG LOADED POLYMERIC MICELLES (Pavan Kumar Reddy *et al.,* 2015)

Drug loaded polymeric micelle can be prepared by following approaches

- Direct dissolution
- Solvent evaporation or film rehydration method and
- Dialysis
- Freeze drying method

Direct dissolution: The simplest technique for preparing drug loaded polymeric micelles is through direct dissolution of amphiphilic copolymer and drug in water. At or above CMC, the copolymer and the drug self-associate in water to form drug loaded micelles. But this method is usually associated with low drug loading. To enhance drug loading this technique can be combined with an increase in temperature or alternatively a thin evaporated film of a drug can be prepared before the addition of copolymer

Solvent evaporation: In this a volatile organic solvent is used to dissolve the copolymer and the drug. A thin film of drug and copolymer is obtained after the solvent is removed by evaporation. Drug-loaded polymeric micelles are obtained by reconstituting the film with water.

Dialysis: This method is used when the two above mentioned techniques are unsuitable as in case when the core forming blocks are long and more hydrophobic. Micelles from such copolymers have more potential to solubilize large amounts of hydrophobic drugs. In these cases, the dialysis technique can be used to prepare drug loaded micelles. Solutions of the drug and the polymer in organic solvent areplaced in the dialysis bag and the solvent is exchanged with water by immersing bag into water thereby inducing micelle assembly. But the dialysis method often requires more than 36 hours for efficient loading.

Freeze drying method: The above mentioned limitations can be overcome by employing a simple and cost effective method in which water/tert-butanol mixture is used for dissolving drug and polymer and then the solution is lyophilized. Drug loaded polymeric micelles are then obtained by redispersing the lyophilized product in suitable vehicle. This method is called Freeze drying method.

ADVANTAGES OF POLYMERIC MICELLES (Pavan Kumar Reddy *et al.*, 2015)

- Very small particle size (10-100nm): This condition is important for selected drug administration routes (such as extravasation into solid tumors, or percutaneous lymphatic delivery). From a practical point of view, the micelles preparation are easy to handle, prepare and sterilize by filtration because of their small size.
- High structural stability
- Large amount of drug loading: Polymeric Micelle carrier system incorporates a large amount of hydrophobic drugs within the inner core and makes them available at the site of interest
- High water solubility: The combined hydrophilic/hydrophobic structure help improve the solubility of poorly water soluble drugs
- Low toxicity: Polymers used to synthesize PMs are known to be less toxic than low-molecular-weight surfactants, such as sodium dodecyl sulphate. PMs are considered very safe in relation to chronic toxicity, as it possess much larger size than that for critical filtration in the kidney, hence it can evade renal filtration. On the other hand, all polymer chains can be dissociated (as single polymer chains) from the micelles over a long time period. This phenomenon results in the complete excretion of the block copolymers from renal route if the polymer chains are designed with a lower molecular weight than the critical value for renal filtration. This is a significant advantage for PMs over the conventional (nonmicelle forming) and nonbiodegradable polymeric drug carrier systems.
- Incorporation of various diagnostic agents: PMs can also be modified to incorporate routinely used diagnostic agents . Most frequently used diagnostic moieties for three major imaging modalities chelated radioactive metals such as ^{111}In or ^{99m}Tc, for scintigraphy; chelated paramagnetic metals such as Gd, for magnetic resonance imaging (MRI) and organic iodine for X-ray computed tomography (CT). Polymeric micelles have been synthesized incorporating all of the above diagnostic agents

DISADVANTAGES OF POLYMERIC MICELLES (Pavan Kumar Reddy *et al.,* 2015)

Though Polymeric micelles have several advantages and are routinely used for drug delivery applications, they suffer from a few limitations that warrants improvement in their design and development to advance this field further. These limitations are: [review, 32]

- Difficult Polymer synthesis

  The maintenance of stability of the polymeric micelles will occur by the process of cross linking via disulphide bridges or radical polymerization etc., but high levels of polymer chemistry are needed in the synthesis of PMs and also in the understanding of the cross linking process. Thus to facilitate micelle formation and ensure colloidal stability and to overcome this limitation the core-forming block needs to be highly hydrophobic while corona forming block needs to be highly hydrophilic.
- Retention of Drugs with in micelles

  In the case of some PMs, the encapsulated drug molecule is not retained within the micelle during circulation. The drug molecules may diffuse from micelles and bind with proteins or cells before they reach the target site.
- Possible chronic liver toxicity due to slow metabolic process

  This is the common limitation of polymeric carriers

APPLICATIONS (Pavan Kumar Reddy *et al.,* 2015)

- **PMs as Diagnostic agents:**

  PMs composed of amphiphilic block copolymers represent a promising class of diagnostic agents. Diagnostic agents can be covalently linked to the water-soluble part of the polymers or incorporated into the water-insoluble core non-covalently. Resulting particles can be used as particulate agents for diagnostic imaging using three major imaging modalities-scintigraphy, magnetic resonance imaging (MRI) and X-ray computed tomography (CT). PMs have been prepared to incorporate ^{111}In or ^{99m}Tc, Gd and organic iodine for usie in scintigraphy, MRI and CT respectively.
- **PMs as Transepithelial Drug Delivery Vehicle:**

  Transepithelial drug delivery can be attained by polymeric micelles because of their ability to internalized into cells and/or cross epithelial barriers, thus the drugs can be deliveres either systematically or locally following non-parenteral administration. There are two possible routes by which intact polymeric micelles pass across epithelial barriers (i) transcellular transport via the process of transcytosis and (ii) paracellular transport between epithelial cells due to their relatively small size and hydrophilic surface.
- **PMs as protein Drug Delivery Vehicle:**

  Nano-sized protein encapsulated PMs was prepared by self assembling human serum albumn (HSA) as a model protein and degradable block copolymer methyl poly(ethylene glycol)-poly(b-amino ester) (PEG-PAE) with piperidine and imidazole rings. The result shows that the albumin encapsulated PEG-PAE-API1-(3-Aminopropyl)

imidazole (API) can be used as a pH-triggered targeting agent and an effective drug delivery system in cerebral ischemia models. Owing to its unique ability of simultaneous acid-triggered targeting and effective delivery of proteins, this strategy may be utilized in the design of general platforms for delivering other proteins in biomedical field

- **PMs in Oncology:**

  The PM size too large for extravasation fron normal vessel walls and renal excretion, and too small from extravasation from blood vessels combined with pathophysiological characteristics of hypervascularity, solid tumor tissues, secretion of vascular permeability factors, incomplete vascular artichitechture and absence of effective lymphatic drainage which leads to enhanced permeability and retention (EPR) effect of PM in solid tumors. Apart from its solubilization, small particle size, long circulation, targeting and easy production properties, PM system can also alter the drug internalization route and subcellular localization, lessen the P-glycoprotein efflux effect, consequently, exert a different mechanism of action from the entrapped drugs.

- **pH-Sensitive Polymeric Micelles for Cancer Chemotherapy:**

  Conventional chemotherapeutic agents used for cancer therapy suffer from multidrug resistance of tumor cells and has poor antitumor efficacy. Development of pH-sensitive polymeric micellar delivery systems is one effective approach to improve the efficacy of cancer chemotherapy because of physiological differences between the tumor tissue and normal tissue. The acid-liable bonds between the therapeutic agents or copolymers with reversible protonation-deprotonation core units and the micelle-forming copolymers can be used to form pH sensitive PMs for extracellular and intracellular drug smart release. pH-sensitive polymeric micelles have been emerging as a fascinating class of nano drug carriers for programmable drug targeting delivery in the foreseeable future.
  E.g. Poly(ethylene glycol)-cis-aconityl-chitosan-stearic acid polymeric micelles-Doxorubicin.

- **Biodegradable PMs for Anticancer Drug Delivery:**

  Biodegradable PMs have emerged as one of the most promising platforms for targeted and controlled anti-cancer drug delivery due to their prolonged circulation time, excellent biocaompatibiliy, enhanced accumulation in tumor, and *in vivo* degradability. Biodegradable micelles are of particular interests for co-delivery of two or more anticancer drugs, which are released either simultaneously or sequentially to achieve synergistic treatment effects (combination cancer therapy). In the future, it is anticipated that biodegradable delivery system will play an important role in clinical cancer treatments. E.g. Block ionomer complexes (BIC) of poly(ethylene oxide)-*b*-polymethacylic acid) (PEO-*b*-PMA) and divalent metal cations (Ca^{2+}) were utilized as templates. Disulfide bonds were introduced into the ionic cores by using cystamine as a biodegradable cross-linker. Here the drug used is doxorubicin.

REVIEW OF LITERATURE- PLURONIC POLYMERIC MICELLES

1. **Liyan Zhao *et al.,*** formulated curcumin loaded mixed micelles to improve the solubility and biological activity of curcumin by thin film hydration method using triblock copolymer Pluronic P123 and F68. The mixed polymeric micelle composed of P123 and F68 with ratio of 2.05:1 exhibited higher entrapment efficiency and drug loading for curcumin. The average size of the curcumin loaded mixed micelles was 68.2 nm and showed sustained release ; and the *in-vitro* cytotoxicity assay showed that Cur-PF micelles presented higher cytotoxic effect on MCF-7 and MCF-7/ADR. Based on these results , it can be concluded that the mixed micelle formulation developed in this study may be considered as a promising drug delivery system for curcumin (Liyan Zhao *et al.,* 2012).
2. **Ivan Pepic *et al.,*** formulated Pluronic F127/L121 mixed micelle system to evaluate it in terms of stability upon dilution in biologically relevant media and to explore the possibility of preparing F127/L121 micelles in a powder form that can be simply reconstituted to an initial freshly made mixed micelle formulation. The mixed F127/L121 micelles were prepared at a relatively high concentration of Pluronics ($1\%^{w}/_{w}$ for both Pluronics) using two different methods (direct dissolution and film rehydration) with an external input of energy (ultrasonication). The size of the optimized micelles was approximately 75nm with a narrow size distribution and also satisfied the stability criteria upon dilution in different biologically relevant media; where it is stable in PBS upon 100-fold dilution for atleast 10 days and in PBS containing bovin serum albumin upon 10 and 50-fold dilution for atleast 48 and 12h respectively. The influence of the type and amount of cryoprotectant on the prevention of F127/L121 micelles aggregation during the freeze-drying and reconstitution processes shows that the use of trehalose ($5\%^{w}/_{w}$) and sucrose ($2.5\%^{w}/_{w}$) with slow and fast freezing process respectively, resulted in a reconstituted product with mostly similar d_h and PDI values of the fresh micelle formulation (Ivan Pepic *et al.,* 2014).
3. **Zhang Wei *et al.,*** formulated Paclitaxel (PTX) loaded mixed micelles to increase its cytotoxix effect by thin film hydration method using triblock copolymer Pluronic P123 and F127. The optimized formulation showed a particle size of about 25nm with Encapsulation ratio >90% and a sustained release behavior and in addition micelle stability studies implied that introduction of Pluronic F127 (33wt%) into the P123 micelle system significantly increased the stability of PTX-loaded micelles. The *in-vitro* cytotoxicity assay on A-549 cells (human lung adenocarcinoma cell line) shows that PTX loaded micelle has higher cytotoxic effect when compared to Taxol injection and free PTX. Therefore it can be concluded that PTX loaded P123/F127 mixed micelles may be considered as an effective anticancer drug deliverysystem for cancer chemotherapy (Zhang Wei *et al.*,2009).
4. **Rania Moataz El-Dahmy *et al.,*** formulated Vincopetine loaded mixed micelles to increase the *in-vivo* mean residence time after IV injection by thin film hydration

technique using triblock copolymer Pluronic L121 and F127. Simple lattice mixture design was for the optimization using Design-Expert software. The optimized formula containing 68%$^{w}/_{w}$ Pluronic L121 and 32%^{w}/w Pluronic F127, had the highest desirability value (0.621), Entrapmentefficiency, Particle size, Polydispersity index and Zeta potential of the optimized formula were 50.74±3.26%, 161.50±7.39nm, 0.21±0.03 and -22.42±1.72mv respectively and shows *in-vitro* sustained release. The *in-vivo* investigation in rabbits, the optimized formula showed a significantly higher elimination half-life and mean residence time than the market product. The study demonstrated that *in-vitro* and *in-vivo* sustainment behavior could be considered as a promising nanocarrier for the Intravenous delivery of the hydrophobic drug; Vincopectine , having rapid elimination rate with low elimination half-life (Rania Moataz El - Dahmy *et al.,* 2014)

5. **Liangcen Chen *et al.,*** formulated Docetaxel loaded mixed micelles to serve as a potential antitumor drug delivery system in Taxol-resistant non-small cell lung cancer by the thin film hydration method using Pluronic P105 and F127 triblock copolymer. A central composite design was utilized to optimize the process , helping to improve drug solubilization efficiency and micelle stability. The average size of optimized mixed micelle was 23nm, with a 92.40% encapsulation ratio, 1.81% drug loading efficiency and *in-vitro* sustained release. The optimal formulation shows high storage stability in lyophilized form, with 95.7% of the drug content remaining after 6 months storage at 4^{o}C. For multidrug-resistant A549/Taxol cells, DTX-loaded P105/F127 mixed micelles displayed noticeable anti-tumor efficacy higher than *in-vitro* Taxotere injection. The study demonstrated that DTX-loaded P105/F127 mixed micelles could significantly increase the blood circulation time of DTX through pharmacokinetic studies where the mixed micelle achieved 1.85 fold longer Mean Residence Time (MRT) in circulation and a 3.82 fold larger area under the plasma concentration-time curve than Taxotere. Therefore, it could be concluded from the results that DTX-loaded P105/F127 mixed micelles might serve as a potential drug delivery system to over come multidrug resistance in lung cancer (Liangcen Chen *et al.,*2013).
6. **Diogo Silva Pellosi *et al.,*** attempted to develop Pluronic micelles delivering the Photodynamic therapy photosensitizers Benzoporphyrin Derivatives (BPD). The BPDA-ring (BPDMA), its regioisomer ring-B (BPDMB) and a BPDMA/BPDMB mixture (BPD-Mixt) were formulated in Pluronic P123 or F127 as well as P123/F127 mixed micelles at two different mass ratios. This nanocarrier system promoted the encapsulation of both A- and B-ring BPD derivatives and their mixture as monomers enhancing photophysical properties and stability in aqueous solutions even in diluted conditions. The *in-vitro* experiments showed photoactivity of BPD-Mixt similar to that of BPDMA which is of utmost interest due to the use of the under explored B-ring derivative. Indeed the expensive separation step of regioisomers is avoided and implies in cost reduction. Based on these preliminary results BPD-Mixt loaded in binary P123/F127 micelles system allies cost reduction and photodynamic efficiency, which stimulates further

development on this nanosystem and may be of clinical interest for cancer photodynamic therapy (Diogo Silva Pellosi *et al.*, 2016).

7. **Yanzuo Chen *et al.*,** formulated methotrexate loaded Pluronic P105/F127 mixed micelle to enhance the antitumor activity of methotrexate (MTX) in multidrug resistanc modulation by thin film hydration method. The optimized formulation displayed suitable particle size (22nm) and distribution, high drug-loading and pH dependent drug release. The MTX cellular uptake in A-549 and KBv MDR cells was much higher in the PF-MTX group compared to MTX. PF-MTX displayed higher antitumor efficacy than free MTX in both MDR cancerous cell lines. The pharmacokinetic studies demonstrated that PF-MTX can significantly increase the blood circulation time of MTX. *In-vivo* real time studies also indicated passive accumulation of polymeric micelles in tumor tissues. Furthermore PF-MTX exhibited remarkable antitumor activity against KBv MDR tumor xenografts and induced less systemic toxicity in comparison with MTX injection. Taken together, PF-MTX micelles are a potential drug delivery system for MDR tumor chemotherapy (Yanzuo Chen *et al.*, 2013).
8. **S. S. Kulthe *et al.*,** developed mixed micelles by varying the ratio of hydrophobic Puronic L81 and relatively hydrophilic Pluronic P123 by taking Aceclofenac (Acl) as a model hydrophobe for drug delivery application. The mixed micelles promise a high solubilization potential for hydrophobic drugs and developed small sized micelle dispersions (~20nm) with fairly high entrapment efficiency, drug loading and low polydispersity indices with sustained release profile for Aceclofenac. The TEM demonstrated spherical shape of micelles. Stable dispersions were obtained for 0.1/1.0wt% and 0.5/3.0wt% Pluronic L81/P123. Micelles were also found to be stable in bovine serum albumin solution. Presence of salt lowered Acl solubilization in micelles. Thermodynamic parameters for Acl solubilization in mixed micelles revealed high partition coefficient values and spontaneity of drug solubilization. Thus the developed novel mixed micelles hold promise in controlled and targeted drug delivery owing to their vey small size, high entrapment efficiency and stability (Kulthe *et al.*, 2011).
9. **Shilpa Praveen Chaudhari *et al.*,** investigated the solubilization of poorly water soluble drug Lamtrigine in pure and mixed pluronic polymeric micelles. The polymeric micelles containing Lamotrigine were prepared by direct dissolution technique using block copolymer (Pluronic L81, Pluronic F68) in combination (1:1) ratio and alone by using various drug:polymer ratios. Mixed micelles (hydrophilic and hydrophobic) helped to overcome the limitations of monosystem of Pluronic L81 and Pluronic F68. Results show that the solubilization of Lamotrigine enhances with the rise in concentration of block copolymers and temperature, but no significant increase was observed with added salt and at a lower pH the drug show highest solubility. In conclusion mixed micelles showed fairly high entrapment efficiency, loading capacity and sustained release profile for Lamotrigine than that of plain pluronic micelles (Shilpa Praveen Chaudari & Jayashree Ramesh Patil, 2014).

10. **Suk Hyug Kwon *et al.,*** formulated genistein loaded pluronic F127 polymeric micelles for oral drug delivery application by solid dispersion method. Drug loading amount and drug loaded efficiency were 11.81% and 97.41% respectively. The average size was 27.76nm and *in-vitro* release was 58% and 82% in pH 1.2 and pH 6.8 respectively at 12 hours. The bioavailability of genistein-loaded polymeric micelles was better than genistein posder. Consequently, Pluronic F127 polymeric micelles are an effective delivery system for the oral administration of genistein (Suk Hyung Kwon *et al.,* 2007).

REVIEW OF LITERATURE – RALOXIFENE HYDROCHLORIDE

1. **Sebastien Taurin *et al.,*** demonstrated the advantages of encapsulating raloxifene into SMA and its cytotoxic potency in two Castrate Resistant Prostate Cancer (CRPC) cell lines differing in the level of ERα and ERβ expression compared to free drug. The SMA-Ral micelles had 132 and 140% higher cytotoxicity against PC3 and DU145 prostate cell lines respectively. SMA-Ral effectively inhibits cell cycle progression, increases apoptosis and alters the integrity of tumor spheroid models. In addition, the micellar system induced changes in expression and localization of estrogen receptors, epidermal growth factor receptor (EGFR) and downstream effectores associated with cell proliferation and survival. Finally, SMA-Ral treatment decreased migration and invasion of CRPC cell lines. In conclusion, SMA-Ral micelles can potentially benefit new strategies for clinical management of Castrate Resistant Prostate Cancer (Sebastien Taurin *et al.,* 2014).
2. **Anand Kumar Kushwaha *et al.,*** prepared Raloxifene loaded Solid Lipid Nanoparticles (SLN) using Compritol 888 ATO as lipid carrier and Pluronic F68 as surfactant by solvent emulsification/evaporation method to improve the oral bioavailability of raloxifene (RL). Particle size of all the formulations were in the range of 250 to 1406nm and the entrapment efficiency ranges from 55 to 66%. *In-vitro* drug release studies were performed in phosphate buffer of pH 6.8 using dialysis bag diffusion technique FTIR and DSC studies indicated no interaction between drug and lipid, and the XRD spectrum showed that RL-Hcl is in amorphous form in the formulation. *In-vitro* release profiles were biphasic in nature and followed Higuchi model of release kinetics. Pharmacokinetics of raloxifene loaded SLN after oral administration to wistar rats was studied. Bioavailability of RL-Hcl loaded SLN was nearly five time than that of pure RL-Hcl (Anand Kumar Kushwaha *et al.,* 2013)
3. **Arpita Patel *et al.,*** formulated Raloxifene Hydrochloride (RLH) loaded liposomes by thin film hydrartion method using 1:1 molar ratio of DSPC : Cholesterol and investigated its uterine targeting efficiency after intravaginal administration. Radiolabelling of RLH was performed with reduced technetium-99m (^{99m}Tc). Binding affinity of ^{99m}Tc- labeled complexes. Biodistribution study was done in New Zealand white female rabbits by

Gamma scintigraphy revealed the preferential uptake of the formulation by uterus when administered vaginally. Spherical and discrete lipososmes of size 119nm were seen in TEM results. Liposomes had high Entrapment Efficiency of 90.96% with Drug Loading of 27.25%$^{w}/_{w}$ compared to plain drug . Liposomes concentrated and retained with in the uterus for a longer period of time. In conclusion, uterine targeting of RLH loaded liposomes indicates its potential to overcome the limitations of marketed formulation. Drug targeting to site of action anticipates dose reduction needed to elicit the therapeutic effect (Arpita Patel *et al.,* 2016).

4. **Manal A Elsheikh *et al.,*** Formulated Raloxifene loaded self-nanoemulsifying drug delivery systems (SNEDDS) to enhance RLX delivery to endocrine target organs. The Raloxifene (RLX) was loaded in the dissolved dispersed status in the alkalinized (A-SNEDDS) and non alkalinized (NA-SNEDDS) systems respectively. *In-vitro* release was assessed using dialysis bag versus dissolution cup methods. NA-SNEDDS were developed with suitable globule size (38.49±4.30), ZP (31.70±3.58mv), PDI (0.31±0.02) and cloud point (85^{o}C). A-SNEDDS exhibited good globule size (35±2.80nm), adequate PDI (0.28±0.06) and lower ZP magnitude (-21.20±3.46mv). TEM revealed spherical globules and contended data of size analysis. Release studes demonstrated a nonsignificant enhancement of RLX release from NA-SNEDDS compared to drug suspension with the lowest release shown by A-SNEDDS. *In-vivo* studies reflected a poor *in-vitro/in-vivo* correlation in solubilized form (A-SNEDDS). In conclusion, NA-SNEDDS possessed superior *in-vitro* characteristics to A-SNEDDS , with equal *in-vivo* potential. NA-SNEDDS elaborated in this work could successfully double RLX delivery to endocrine target oragans, with promising consequences of lower dose and side effects of the drug (Manal A Elsheikh *et al.,* 2012).
5. **Jaya Prakash Shanmugam, Santhiagu Arockiasamy and Jasemine** formulated raloxifene loaded gellan gum nanoparticles to develop a better system to deliver poorly water soluble hydrophobic drugs like raloxifene Hcl (RLX-Hcl) by emulsion cross linking method. The developed nanoparticles showed narrow particle size distribution with an average size of 472nm, zeta potential of -40.6mv along with 98±3% entrapment efficiency. FTIR studies demonstrated chemical interaction between the polymer and drug. *In-vitro* release studies showed an initial release within 30 min followed by continuous release for 24 hours. *In-vitro* cytotoxicity studies performed with MCF7 cell line revealed that the RLX Hcl-gellan gum nanoparticles exhibit higher cytotoxicity compared to free RLX Hcl. The result suggest that gellan gum nanoparticle system can be a better system to deliver hydrophobic drug like Raloxifene Hydrochloride (Jaya Prakash Shanmugam, Santhiagu Arockiasamy and Jasemine *et al.*, 2014).
6. **Tuan Hiep Tran *et al.,*** demonstrated the study to improve the physicochemical properties and bioavailability of a poorly water-soluble drug, raloxifene by Solid Dispersion (SD) nanoparticles using the spray-drying technique. These spray dried SD nanoparticles were prepared with raloxifene (RXF), poly vinyl pyrrolidone (PVP) and

Tween 20 in water. Reconstitution of optimized RXF-loaded SD nanoparticles in pH 1.2 medium showed a mean particle size of approximately 180nm.X-ray diffraction and differential scanning colorimetry indicated that RXF existed in amorphous form with in spray-dried nanoparticles. The optimized formulation showed an enhanced dissolution rate of RXF at pH 1.2, 4.0, 6.8 and distilled water as compared to pure RXF powder. The improved dissolution of raloxifene from spray-dried SD nanoparticles appeared to be well correlated with enhanced oral bioavailability of raloxifene in rats. Furthermore, the pharmacokinetic parameters of the spray dried SD nanoparticles showed increased $AUC_{0-\infty}$ and C_{max} of RXF by approximately 3.3 fold and 2.3 fold respectively. These results suggest that the preparation of RXF-SD nanoparticles using the spray drying technique without organic solvents might be a promising approach for improving the oral bioavailability of RXF (Tuan Hiep Tran *et al.*,2013).

7. **Ashok Velpula *et al.,*** investigated the feasibility of neutral and charged proliposomes for the improved oral delivery of RXH. RXH could be loaded into proliposome formulation by film deposition method using spray dried mannitol as carrier. The effect of surface charge was studied by tailoring of optimized formulation (RXH- PL3) with diacetyl phosphate and stearyl amine. The solid state characterization unravels the transformation of crystalline state of RXH to amorphous and /or molecular state. The predicted effective permeability coefficient and fraction dose absorbed in humans were much higher for anionic and cationic charged proliposomes compared to RXH-PL3 and control formulation. A two to three fold improvement in the bioavailability of RXH reveals the potential of proliposome formulation and the importance of surface charge on the preferential uptake across the GI barrier (Ashok Velpula *et al.,* 2013).
8. **Bhama , S., Sambath Kumar, R. S., and Rajagopal Shanmuga Sundaram** formulated RLX-Hcl loaded proniosomes by slurry method, where different ratios of surfactant and cholesterol were used for preparation and evaluating study. The shape of prepared RLX-Hcl loaded proniosomes were spherical with an average particle size of 690nm with a high encapsulation efficiency of 83.64% and *in-vitro* drug release of 99.42% were attained after 24 hrs. The vesicles were quite stable at $5^{o}C$ over a period of 90 days. Hence it may be concluded that proniosome formulation proved as efficient carrier for raloxifene oral delivery (Bhama, Sambath Kumar & Rajagopal Shanmuga Sundaram, 2016).
9. **Dimitrios Bikiaris, Vassilios Karavelidis and Evangelus Karavas** formulated Raloxifene Hcl nanoparticle using biodegradable polymers. For this purpose a series of novel biodegradable poly (ethylene succinate) (PESu) and poly (propylene adipate) (PpAd) were used. The prepared polyesters were characterized by intrinsic viscosity measurements, end group analysis, enzymatic hydrolysis, Nuclear Magnetic Resonance Spectroscopy (^{1H}NMR and ^{13}C-NMR) and wide angle X-ray Diffractometry (WAD). From the characterization of the copolyesters it was found that only P(ESu-co-PAd) 90/10, 80/20 and 70/30 were in crystalline form, while all the others remained

amorphous. The melting point of the P (ESu-co-PAd) 70/30 is lower from the melting point of PpAd. These two polymers characterized as thermosensitive polymers and could be appropriate for targeting delivery system. The Raloxifene Hcl nanoparticles have been prepared by variation of the coprecipitaion method. WAXD showed that Raloxifene Hcl is entrapped in crystalline form and possibility in nanocrystalline shapes within the nanoparticles. The particle size distribution showed that the nanoparticles are in the range of 200-350nm. It was found that the size of the nanoparticles is higher for the polymers with higher content in PAd. The drug release rates from the prepared polyesters are very low. It seems that these results depend on the drug's crystallinity within the nanoparticles as well as on the melting point of used polyesters. Finally, it was found the nanoparticles with higher particle size mentioned higher release rates (Dimitrios Bikiaris, Vassilios Karavelidis and Evangelus Karavas, 2009).

10. **Deepa Saini *et al.,*** formulated Raloxifene loaded chitosan Nanoparticles for the treatment of osteoporosis with enhanced bioavailability by gelation of chitosan tripolyphosphate (TPP) and then by ionic cross-linking. Formlation was optimized and *in-vitro* drug release and *in-vivo* study were performed. The particle size, entrapment efficiency and loading efficiency varied from 216.65 to 1890nm, 32.84 to 97.78% and 23.89 to 62.46% respectively. Release kinetics showed diffusion-controlled and Fickian release pattern. *In-vivo* study indicated higher plasma drug concentration with nanoparticles administered intranasally as compared to drug suspension administered through oral route ($P<0.05$). A significant higher drug concentration in plasma was achieved in 10min after nasal administration with respect for oral administration. In conclusion, the results suggest that RLX-loaded chitosan Nanoparticles have better bioavailability and would be a promising approach for intranasal delivery of Raloxifene for the treatment of osteoporosis (Deepa Saini *et al.*, 2015).

AIM OF THE WORK

- The aim of the present study is to formulate Raloxifene hydrochloride Polymeric Micelle drug delivery system using mixture of triblock copolymers, Pluronic L121 and F127 by thin film hydration method.

OBJECTIVE

- To provide an efficient dosage form to improve the therapeutic efficacy by protection of Raloxifene hydrochloride (RXH) from extensive first pass metabolism (Authority of the Board of the American Society of Health System, 2011) and thereby improving the bioavailability.
- To overcome its limitation of poor aqueous solubility (Helga Handottir, 2008).

PLAN OF WORK

- Drug excipients compatibility studies- FT-IR study
- Determination of λ_{max} of Raloxifene Hydrochloride in Phosphate buffer pH 6.8
- Calibration curve of the drug in phosphate buffer pH 6.8
- Formulation of Raloxifene Hydrochloride Pluronic Polymeric Micelles using optimized formulation parameters with different concentrations of hydrophilic (Pluronic F127) and hydrophobic (Pluronic L121) copolymers
- Evaluation of Raloxifene Hydrochloride Mixed Pluronic Polymeric Micelles
  - Entrapment Efficiency
  - *In-vitro* drug release study using pH 6.8 phosphate buffer containing $0.5\%^{v}/_{v}$ polysorbate 80 by dialysis bag method
  - *In-vitro* release kinetics
- Determination of particle size distribution, polydispersity index (PDI) and zeta potential analysis using Malvern zeta analyzer
- Selection of best formulation
- Concentration of best forulation by lyophilization using cryoprotectant ($2.5\%^{w}/_{w}$ sucrose)
- Morphological studies using Confocal Microscopy

RATIONALE OF THE STUDY (Ernico M. Messali & Conoscaffa, 2009)

Osteoporosis is a systemic skeletal disease characterized by low bone mass and microarchitectural deterioration of bone tissue, with a consequent decrease in bone mineral density (BMD) and increase in bone fragility and susceptibility to fracture.

The decreased estrogen circulating level during postmenopausal age represents the main cause of bone loss and osteoporosis and about 54% of women aged 50 years or older will have an osteoporotic fracture during their lifetime. A rapid decrease of bone mass is evident in the first 5 to 10 years following the menopause and the annual rate of bone loss is at a maximum of about 4% during the former 4 years, then declines to 1%. In this period, the physiological bone remodeling is characterized mainly by a relevant prevalence of the resorption due to osteoclastic activity.

There are several drugs to treat postmenopausal Osteoporosis but adherence to osteoporosis medications is relatively poor approximately 20% to 30% of patients taking daily or weekly treatments may suspend their treatment within 6 to 12 months of initiating therapy. The majority of patients who discontinue therapy appear to do so because of drug induced adverse effects and fear of health risks. Asian women showed a greater propensity to remain on raloxifene, compared with bisphosphonates, and the women on raloxifene exhibited lower discontinuation rates and higher treatment satisfaction, which addressed the more favorable compliance and tolerance with raloxifene than with bisphosphonates. Postmenopausal women with osteoporosis, who have poor compliance when taking alendronate, can be switched to raloxifene, because they can still see benefi ts in BMD and bone turnover with raloxifene after discontinuing alendronate therapy.

RATIONALE FOR SELECTION OF DRUG

Raloxifene hydrochloride is a non-steroidal selective estrogen receptor modulator (SERM) which has marketed for use in prevention and treatment of postmenopausal osteoporosis. One of the consequences of the Women's Health Initiative has been increased interest in the SERMs, because of the potential to retain most of the beneficial effects of estrogen while avoiding some of the adverse effects. Raloxifene binds to estrogen-receptors with estrogen agonistic effects in some tissues and estrogen antagonistic effects in others. In the last few years, a number of clinical studies have been published on the effects of raloxifene on osteoporosis, the risk of invasive breast cancer and cardiovascular diseases. There are a number of other SERMs currently under investigation but raloxifene is the only SERM currently on the market for osteoporotic fractures (Helga Handsdottir, 2008).

Raloxifene hydrochloride (RXH) is an orally selective estrogen receptor modulator (SERM) with poor bioavailability due to its poor aqueous solubility and extensive first pass metabolism where approximately 60% of an oral dose is absorbed , but presystemic

glucuronide conjugation is extensive. Absolute bioavailability as unchanged raoxifene is 2.0% (ASHP, 2011).

To overcome these problems and in order to improve the oral bioavailability of raloxifene, RXH loaded polymeric micelle have been developed using pluronic L121and pluronic F127.

RATIONALE FOR SELECTION OF POLYMERIC MICELLAR FORMULATION

The purpose of this research work was to formulate and optimize the Pluronic F127/L121 mixed micelle system containing RXH to enhance its solubility and therapeutic efficacy by protection from extensive first pass metabolism through reduced liver uptake. This can be accomplished by pluronic triblock copolymer whose poly (ethylene oxide) block are sterically stabilized (stealth) and undergo less opsonization and uptake by the macrophages of the reticuloendothelial system (RES). Another advantage of using pluronic is that, even though PEO-PPO-PEO materials are non degradable molecules with a molecular weight in 10-15 KDa range are usually filtered by the kidney and cleared in urine (Diego Chiapetta & Alejandro Sosnik, 2007). The rationale of using mixture hydrophilic (pluronic F127) and hydrophobic (pluronic L121) copolymer is that the hydrophobic pluronic L121 have a relatively high solubilization capacity but the micelles formed are large and unstable whereas the hydrophilic pluronic F127 form spherical micelles and have a high stability but they have a relatively low solubilization capacity. Thus pharmaceutical nanocarriers based on pluronic mixtures may overcome the aforementioned deficiencies while improving the solubilization capacity and stability of the micelles than that composed of the individual pluronics (Ivan Pepic et al., 2014).

Pluronic polymeric micelles have been reported to offer the following advantages (Diego Chiapetta & Alejandro Sosnik, 2007).

- Solubilization of water insoluble molecules
- Protection of unstable agents from chemical degradation and metabolism by biological agents
- Sustained release

Osteoporosis is a disease that weakens bones, increasing the risk of sudden and unexpected fractures. Literally meaning "porous bone," osteoporosis results in an increased loss of bone mass and strength. The disease often progresses without any symptoms or pain. Postymenopausal osteoporosis has a direct relationship between the lack of estrogen during perimenopause and menopause and the development of osteoporosis. Early menopause (before age 40) and any prolonged periods in which hormone levels are low and menstrual periods are absent or infrequent can cause loss of bone mass. Estrogen deficiency plays a role in the pathogenesis of postmenopausalosteoporosis and of the mechanism of estrogen actionin bone has grown considerably. This is mainly a result of the recognition that estrogen regulates bone remodeling by modulating the production of cytokines and growth factors from bone marrow and bone cells (WebMd L.L.C, n.d.). Postmenopausal osteoporosis should be regarded as the product of an inflammatory disease bearing many characteristics of an organ-limited autoimmune disorder, triggered by estrogen deficiency, and brought about by chronic mild decreases in T cell tolerance (Neale Weitzmann & Roberto Pacifici, 2006).

PATHOGENESIS AND PATHOPHYSIOLOGY OF POSTMENOPAUSAL OSTEOPOROSIS (Nelson Watts *et al.,* 2010)

Low bone mass and skeletal fragility in adults may be the result of low peak bone mass in early adulthood, excessive bone loss in later life, or both. The skeleton is constantly changing throughout life. During childhood and adolescence, it changes in size, shape, and constituents by a process known as modeling.Change in shape and size is complete with epiphyseal closure at the end of puberty, followed by a period of consolidation for 5 to 10 years (depending on the skeletal site) until peak adult bone mass is attained, which usually occurs in the late teens or early 20s. Approximately 70% to 80% of peak bone mass is genetically determined. Several genetic markers have been identified. Many nongenetic factors contribute, including nutrition (for example, calcium, phosphate, protein, and vitamin D), load-bearing activity, and hormones involved in growth and puberty.

Once peak adult bone mass has been reached, a process called skeletal remodeling takes over, in which old bone is replaced by new bone. Remodeling is governed by the actions of osteoclasts that resorb old bone and osteoblasts that produce new bone. Much is known about the recruitment and activity of these cells, including the involvement of systemic hormones and local cytokines. Recently, the receptor activator of nuclear factor-kb (RANK), its ligand RANKL, and a decoy receptor, osteoprotegerin (OPG), have emerged as major local regulators of bone remodeling. RANKL, synthesized by osteoblasts and stromal cells and present in the bone microenvironment, binds to RANK, expressed in osteoclast progenitor cells in the bone marrow, and promotes osteoclastogenesis. OPG is also synthesized by osteoblasts and stromal cells and serves as a decoy receptor for RANKL, preventing the binding of RANKL to RANK. Regulation of osteoclast activity depends, at least in part, on the balance between RANKL and OPG. The relative amount of these 2 molecules is governed, in turn, by systemic hormones (for example, estrogen), local factors (such as interleukin-6 and tumor necrosis factor), and perhaps other factors as well. The triggering mechanisms that stimulate the cascade of activities that lead to remodeling of site-specific quantities of bone are not known. It is well documented, however, that this bone remodeling process is in balance (that is, the rate of bone formation equals the rate of bone resorption) through at least the fifth decade of life in healthy individuals.

In women, the hormonal changes that occur throughout perimenopause and the immediate postmenopausal years stimulate RANKL production (both directly and indirectly),

leading to accelerated bone loss. Most data suggest that the bone turnover rate (and bone loss) accelerates 3 to 5 years before the last menstrual period and slows again 3 to 5 years after the last menstrual period. With the accelerated bone turnover rate, bone balance is disturbed because there is greater net loss than gain in each of the bone remodeling units that are activated. The mean rate of bone loss during this period is about 1% per year, or about 10% during the menopausal transition.

EFFECTS OF ESTROGEN DEFICIENCY ON BONE TURNOVER AND ARCHITECTURE:

Aging bone is gradually replaced by new tissue through a process called bone remodeling or turnover. Bone remodeling occurs through the coordinated action of OBs and OCs. The activities of OCs and OBs are combined into defined anatomical spaces called basic multicellular units (BMUs). A remodeling cycle begins with the activation of a new BMU on a previously inactive surface of bone. This process involves the disappearance of bone-lining cells and their replacement by OCs that generate resorption lacunae on the endosteal surface of bone over a 2-week interval. The resorption phase is then terminated, probably by OC apoptosis, and after a brief reversal phase, a team of OBs is recruited that fills in the resorption cavity with new bone. The net result is the replacement of a packet of old bone with new bone. At menopause there is a transient, accelerated phase of bone loss that is followed by slower, sustained bone loss. Although in men there is no abrupt cessation of gonadal function in the sixth decade of life, they do experience an age-related decrease in unbound sex steroids resulting from progressive increases in circulating sex hormone–binding globulin. In both men and women there is a steady decline in unbound (bioavailable) estrogen levels with aging, exacerbated in women at menopause by a marked decrease in estrogen levels (Nelson Watts *et al.,* 2010).

Estrogen deficiency leads to dramatic elevations in the number of BMUs through increased activation frequency, which is the number of new remodeling units activated in each unit of time. Enhanced activation frequency expands the remodeling space, increases cortical porosity, and enlarges the resorption area on trabecular surfaces. This phenomenon is caused primarily by increased OC formation, a complex event involving various hematopoietic and immune cells, as well as increased OC recruitment to bone surfaces to be remodeled. Estrogen deficiency also augments erosion depth by prolonging the resorption phase of the remodeling cycle through increased OC lifespan due to reduced apoptosis. The net bone loss caused by the combined effects of increased activation frequency and erosion depth is limited in part by a compensatory augmentation of bone formation within each remodeling unit. This event is a consequence of stimulated osteoblastogenesis fueled by an expansion of the pool of early mesenchymal progenitors and by increased commitment of such pluripotent precursors toward the osteoblastic lineage. In spite of stimulated osteoblastogenesis, the net increase in bone formation is inadequate to compensate for enhanced bone resorption because of an augmentation in OB apoptosis, a phenomenon also induced by estrogen deficiency (Nelson Watts *et al.,* 2010).

An additional event triggered by estrogen withdrawal, which limits the magnitude of the compensatory elevation in bone formation, is the increased production of inflammatory cytokines such as IL-7 and TNF, which limit the activity of mature OBs. Increased bone resorption, trabecular thinning and perforation, and a loss of connection between the remaining trabeculae are the dominant features of the initial phase of rapid bone loss that follows the onset of estrogen deficiency. This acute phase is followed by a long-lasting period of slower bone loss

where the dominant microarchitectural change is trabecular thinning. This phase is due in part to impaired osteoblastic activity secondary to increased OB apoptosis (Nelson Watts *et al.*, 2010).

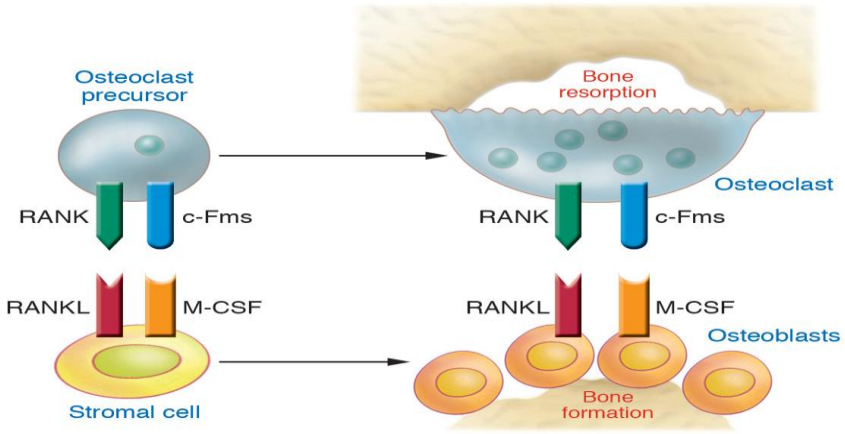


Figure 3: Cells and cytokines responsible for physiological OC renewal. OC precursors may differentiate from the population of monocytes/macrophages, among which they circulate by virtue of their expression of the receptor RANK. When RANKL binds to this receptor in the presence of the trophic factor M-CSF, which in turn binds to its receptor, colony-stimulating factor receptor 1 (c-Fms), OC precursors differentiate and fuse together to form mature, multinucleated bone-resorbing OCs. Under physiological conditions the dominant source of RANKL and M-CSF in the bone marrow microenvironment is from the bone-forming cells, the OBs, and their SC precursors (Neale Weitzmann & Roberto Pacifici, 2006).

TREATMENT

- ✓ Estrogen Replacement Therapy
- ✓ Selective Estrogen Receptor Modulators
- ✓ Bisphosphonates
- ✓ Calcitonin
- ✓ Parathyroid hormone
- ✓ Other treatment

Estrogen replacement therapy (Neale Weitzmann & Roberto Pacifici, 2006)

Initiation of estrogen replacement therapy (ERT) in experimental animals and humans decreases erosion depth and OC activation frequency by stimulating apoptosis and blocking osteoclastogenesis. Long-term ERT at high doses not only blunts bone resorption but also stimulates bone formation, leading to a net anabolic effect. A decrease in OB apoptosis resulting from a nongenotropic effect of estrogen is likely a major mechanism driving this effect. However, the increase in OB lifespan is offset in part by a repressive effect of estrogen on osteoblastogenesis, a phenomenon that explains why the anabolic effect of estrogen is observed only at high doses and during long-term treatment

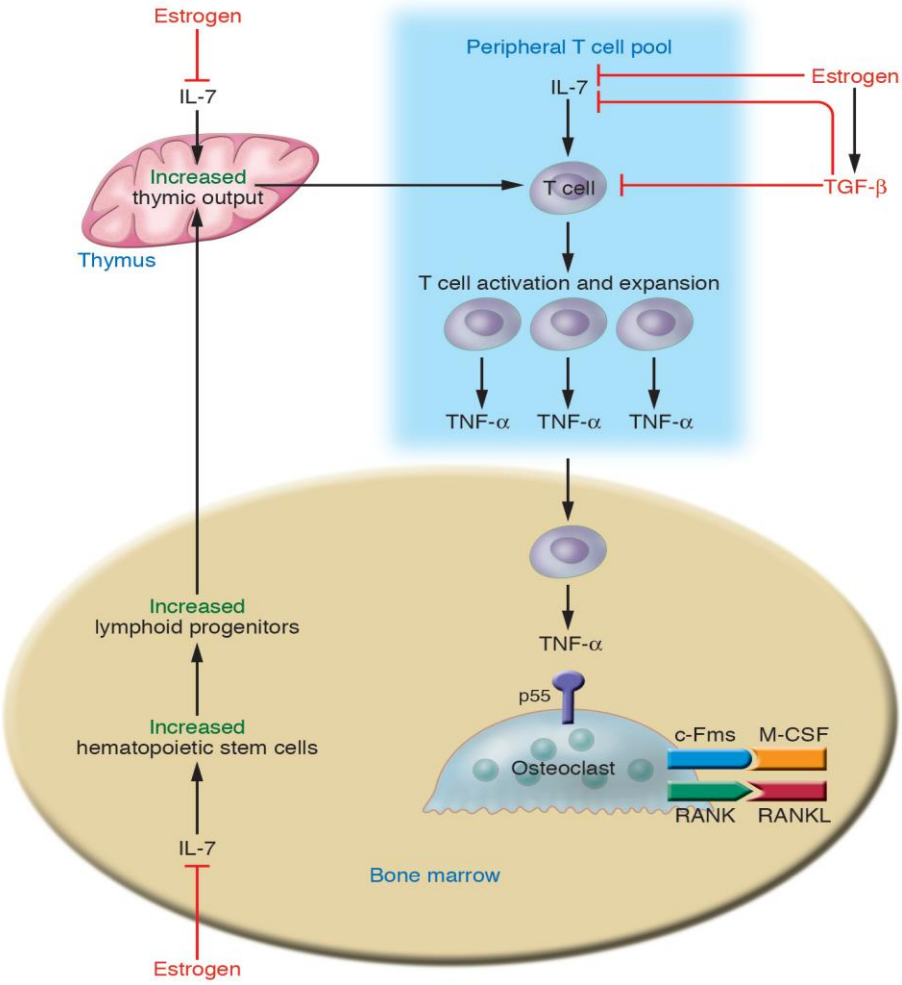


Figure 4: Estrogen suppresses T cell TNF production by regulating T cell differentiation and activity in the bone marrow, thymus, and peripheral lymphoid organs. In the bone marrow, estrogen downregulates the proliferation of hematopoietic stem cells through an IL-7–dependent mechanism, resulting in a smaller pool of lymphoid progenitors. T cell precursors leave the bone marrow and migrate to the thymus, where T cell differentiation, selection, and expansion take place, in large measure under control of IL-7. Following release from the thymus (thymic output), these new T cells home to peripheral lymphoid organs, including the bone marrow itself. Estrogen prevents T cell activation in part by directly blunting antigen presentation and in part via repression of IL-7 and IFN-γ production. This effect is amplified by the upregulation of the IL-7 suppressor TGF-β. The net result of these actions is a decrease in the number of TNF-producing T cells. The blunted levels of TNF diminish RANKL-induced OC formation, ultimately preventing bone loss.

Table 1: Benefits and risk of long term hormone replacement therapy in postmenopausal women (Perrie Delmas, 2002)

Degree of evidence	Benefits	Risks
Strong	Relief of menopausal symptoms, Prevention of bone loss	Vaginal bleeding, Breast tenderness ,Deep vein thrombosis and pulmonary embolism
Moderate	Prevention of fractures	Increased risk of breast cancer after long-term use
Weak	Primary prevention of chronic heart disease Improvement of cognitive function, and preventionof Alzeihmer's disease	Slight increased risk of endometrial cancer, Slight increased risk of ovarian cancer

Selective Estrogen Receptor Modulators (SERMs) (Perrie Delmas, 2002)

SERMs and other oestrogen analogues act as oestrogen agonists or antagonists, dependent on the target tissue.
e.g., Tamoxifen, Raloxifene,

- **Tamoxifen**, which has long been used as an adjuvant treatment in breast cancer, is an oestrogen antagonist in breast tissue but a partial agonist in bone, cholesterol metabolism, and the endometrium. Tamoxifen does not wholly prevent bone loss in postmenopausal women but it does increase the risk of endometrial cancer, precluding its widespread use in healthy postmenopausal women.

- **Raloxifene,** is a benzothiophene that competitively inhibits the action of oestrogen in the breast and the endometrium, and that acts as an oestrogen agonist on bone and lipid metabolism. In early postmenopausal women, raloxifene prevents postmenopausal bone loss at all skeletal sites, reduces markers of bone turnover to premenopausal concentrations, and reduces serum cholesterol concentration and its LDL fraction without stimulating the endometrium. Results of the MORE study (Multiple Outcomes of Raloxifene Evaluation), which involved 7705 women with osteoporosis, indicate that a 30% and 50% reduction of incident vertebral fractures in women with and without prevalent vertebral fractures, respectively, happens after treatment with raloxifene. However, no effects on non-vertebral fractures were noted (table). In the MORE study, raloxifene also lowered the frequency of breast cancer by 70%. Raloxifene, can result in a reduction in coronary heart disease in the high-risk population of postmenopausal women as shown in the MORE study. Raloxifene does not impair cognitive function in postmenopausal women and might even slow age-related cognitive deterioration; raloxifene-treated women did better than controls in two tests of verbal memory and attention. Although rare, thromboembolic disease (venous thrombosis and pulmonary embolism) is increased in individuals treated with raloxifene, with a relative risk similar to that seen in those who take HRT.

Bisphosphonates (Perrie Delmas, 2002)

Bisphosphonates are stable analogues of pyrophosphate characterised by a phosphorus-carbon-phosphorus (P-C-P) bond. By substituting for hydrogens on the carbon atom,various bisphosphonates have been synthesised, the potency of which depends on the length and structure of the side chain. Bisphosphonates have a strong affinity for bone apatite, which is the basis for their clinical use. They are potent inhibitors of bone resorption, reducing the recruitment and activity of osteoclasts and increasing their apoptosis through a molecular mechanism recently identified. The oral bioavailability of bisphosphonates is low, between 1% and 3% of the dose ingested, and is impaired by food, calcium, iron, coffee, tea, and orange juice. These drugs are quickly cleared from plasma, with about 50% deposited in bone and 50% excreted in urine. The half-life of bisphosphonates in bone is several years. The safety profile of bisphosphonates is favourable; mild to moderate gastrointestinal discomfort that rarely results in discontinuation of medication has been reported for all (dyspepsia, abdominal pain, diarrhoea).

e.g., alendronate, etidronate, risedronate, clodronate

Calcitonin (Perrie Delmas, 2002)

Calcitonin, a peptide produced by thyroid C cells, reduces bone resorption by direct inhibition of osteoclast activity. When given subcutaneously or intramuscularly, tolerance is sometimes poor (nausea, facial flushes, diarrhoea), whereas intranasal administration of salmon calcitonin has no significant side-effects. The minimum intranasal dose needed for a significant effect on BMD is 200 IU daily. Calcitonin is less effective in prevention of cortical bone loss than cancellous bone loss in postmenopausal women.

Parathyroid hormone (PTH) (Perrie Delmas, 2002)

Excess secretion and continuous intravenous infusion of PTH result in increased bone resorption and bone loss. By contrast, there is compelling evidence, in a range of species made osteoporotic by gonadectomy, that intermittent PTH injection restores bone strength by stimulation of new bone formation at the periosteal (outer) and endosteal (inner) bone surfaces, thickening the cortices and existing trabeculae of the skeleton, and perhaps increasing trabecular numbers and their connectivity.

Other treatments (Perrie Delmas, 2002)

Alfacalcidol and calcitriol are vitamin D analogues and are used in some countries for the treatment of osteoporosis. Both compounds induce a small increase in BMD that seems to be limited to the spine

Non-Pharmacological Intervention (Perrie Delmas, 2002)

- ✓ Nutrition
- ✓ Exercise
- ✓ Orthopaedic Management of Fractures
- ✓ Other measures

Nutrition

Good nutrition and a balanced diet with adequate caloriesare important for normal growth. Calcium is the most important nutrient for attaining adequate peak bone mass,
but there is no universal consensus about the daily calcium requirement by age. The 1994 consensus development conference on optimum calcium intake recommended 1200–1500 mg daily for adolescents, 1000 mg daily for adults up to age 65 years, and 1500 mg daily for postmenopausal women not receiving oestrogen and for elderly people. A recent National Institute of Health panel has reinforced the importance of adequate calcium intake. Although results of most studies indicate a beneficial effect of calcium supplementation, the longterm effect of a high dietary calcium intake on bone health is unclear. Conversely, there seems to be a threshold of calcium intake, around 400 mg per day, below which increasing calcium intake seems beneficial and necessary, both in children and in women older than age 60 years. Vitamin D is essential for the intestinal absorption of calcium and, as discussed above, serum concentrations of 25-hydroxyvitamin D decline with age. Findings of several studies suggest that the daily intake of vitamin D should be around 400–800 IU if sunlight exposure is low. An adequate protein intake is essential in frail elderly individuals.

Exercise:

Physical activity early in life contributes to high peak bone mass. Various activities, including walking, weight training, and high impact exercises, induce a small (1–2%) increase in BMD at some but not all skeletal sites, that is not sustained once the exercise programme is stopped. Fitness might indirectly protect individuals from fractures by improving mobility and muscle function, and by reducing the risk of falls. Findings of observational studies suggest that regular
exercise and recreational activity reduce hip and leg fracture risk but increase the risk of wrist fracture. After a vertebral fracture, a supervised exercise programme to maintain strength and flexibility of the thoracic and lumbar spine is recommended in elderly individuals.

Orthopaedic management of fractures:

Early surgical management of hip fractures is essential to decrease mortality rate and to improve perioperative morbidity, which is pronounced—especially in frail elderly individuals. The surgical treatment of peripheral fragility fractures does not require specific procedures, since the rate at which fracture heals is much the same in patients of a similar age with and without osteoporosis. In patients with major pain related to a crushed vertebra, vertebral plasty, involving injection of polymetalmetacrylate cement into the vertebral body, has been suggested. Although this procedure might have a beneficial effect on acute pain, the long-term effects on the subsequent risk of fractures of adjacent vertebrae have not been assessed.

Other measures

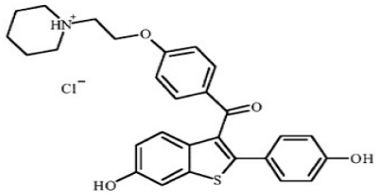
Treatments that predispose to osteoporosis e.g., chronic corticosteroid therapy should be avoided whenever possible. In addition to an exercise programme, a strategy to decrease the risk of falls in elderly individuals should be implemented. Visual impairment and cataract should be detected and treated and whenever possible, the use of drugs that increase the risk of falling e.g., benzodiazepine, hypnotics, antidepressants, and medications that induce hypotension should be reduced. Furthermore, patients should be instructed to avoid slippery floors and install adequate lighting at home. Finally, results of two controlled studies done in elderly individuals in care

homes have shown that the risk of hip fracture could be reduced as much as 50% by use of energy-absorbing external hip protectors. However, long-term adherence with these devices is unknown.

RALOXIFENE HYDROCHLORIDE

PHYSIOCHEMICAL PROPERTIES

Structure (Wishart *et al.*, 2006)**:**



Molecular formula **:** $C_{28}H_{27}NO_4S$. HCl (Medicines and Healthcare products Regulatory Agencies, 2013).

Molecular weight **:** 510.0 (MHRA, 2013).

Chemical Name **:** [6-Hydroxy-2-(4-hydroxyphenyl)-1-benzothiophen-3-yl][4-[2-(piperidin-1-yl)ethoxy]phenyl]methanone hydrochloride (MHRA, 2013).

CAS Number **:** 82640-04-8 (MHRA, 2013).

Category **:** A second generation selective oestrogen receptor modulator (SERM) used to prevent and treat osteoporosis in postmenopausal women. It has estrogen agonist action on bone and cholesterol metabolism but behaves as a complete antagonist on mammary gland and uterine tissue (Wishart *et al.*, 2006).

Description **:** Almost white or pale-yellow powder (MHRA, 2013).

Solubility **:** Freely soluble in dimethylsulphoxide, sparingly soluble in methanol, slightly soluble in ethanol, very slightly soluble in water and isopropyl alcohol and practically insoluble in ether and ethyl acetate (Ministryof Health and Family Welfare, 2014).

Storage **:** Store protected from moisture (ASHP, 2011).

Melting point **:** 258^{o}C (Jo Watts *et al.*, 2011).

INDICATION

For the prevention and treatment of osteoporosis in postmenopausal women (ASHP, 2011)

DOSE

60mg once daily (ASHP, 2011)

PHARMACOKINETICS (ASHP, 2011)

- **Absorption:** Raloxifene hydrochloride is rapidly absorbed from the GI tract. Because raloxifene undergoes extensive first-pass glucuronidation, oral bioavailability of unchanged drug is low. While approximately 60% of an oral dose is absorbed, absolute bioavailability as unchanged raloxifene is only 2%. However, systemic availability of raloxifene may be greater than that indicated in bioavailability studies because circulating glucuronide conjugates are converted back to parent drug in various tissues.
- **Protein Binding:** Raloxifene and its monoglucuronide conjugates are more than 95% bound to plasma proteins. Raloxifene binds to albumin and α_1-acid glycoprotein, but not to testosterone-estradiol binding globulin (sex hormone binding globuli
- **Metabolism:** Hepatic, raloxifene undergoes extensive first-pass metabolism to the glucuronide conjugates: raloxifene-4'-glucuronide, raloxifene-6-glucuronide, a 6, 4'-diglucuronide. No other metabolites have been detected, providing strong evidence that raloxifene is not metabolized by cytochrome P450 enzymes.
- **Elimination $T_{1/2}$:** 27.7hr
- **Route of elimination:** Raloxifene is primarily excreted in feces, and less than 0.2% is excreted unchanged in urine.
- **Volume of distribution:** 2348L/kg (oral administration of single doses rangin from 30 to 150mg)
- **Clearance:** Apparent oral clearance of the drug is 44.1L/kg.hr

PHARMACODYNAMICS (ASHP, 2011)

- Prevention and treatment of osteoporosis in postmenopausal women
- Prevention or treatment of corticosteroid-induced hypogonadism and osteoporosis
- Reduction in the incidence of breast cancer

Prevention and treatment of osteoporosis in postmenopausal women:

Osteoporosis, a systemic skeletal disease characterized by low bone mass and microarchitechtural deterioration of bone tissue with consequent increased bone fragility and

susceptibility to fracture, is observed in a large proportion of 3postmenopausal women. Adult women have less bone mass than men at all ages, and decreased production of estrogen at menopause is associated with accelerated bone loss, particularly from the lumbar spine , for about 5 years, during which skeletal mass loss averages 3% per year. While the risk of postmenopausal osteoporosis is associated with many risk factors including premature ovarian failure; a family history of osteoporosis; a small, slim body frame; endocrine disorders such as thyrotoxicosis, hyperparathyroidism, cushing's syndrome, hyperprolactinemia, and insulin-dependent diabetes mellitus (type I, IDDM); cigarette smoking, drinking excessive amounts of alcohol; a sedentary life style and/or lack of physical exercise; low body weight; and low dietary calcium intake. Premature ovarian failure (surgical or nonsurgical) hastens the onset of osteoporosis, and estrogen deficiency in premenopausal women induces bone loss and may reduce peak bone mass.

Raloxifene is used for the treatment of postmenopausal osteoporosis. Estrogen replacement therapy is effective for the treatment of osteoporosis in postmenopausal women and has recommended as first line therapy for women with osteoporosis. However, because results of a recent controlled study indicate that estrogen/ progestin therapy is associated with a small increase in the risk of breast cancer, cardiovascular disease, stroke, and venous thromboembolism, recommendations on appropriate use of such therapy are being revised. Other therapeutic modalities for the treatment of osteoporosis include alendronate, calcitonin, calcium, risedronate, and vitamin D. Therapy, with alendronate reduces spine and nonspine fracturerates in women with osteoporosis. While recent evidence indicates that raloxifene reduces the risk of vertebral fracture in women with osteoporosis.

Prevention or treatment of corticosteroid-induced hypogonadism and osteoporosis:

Patients receiving long-term corticosteroid therapy may develop hypogonadism secondry to inhibition of secretion of leutinizing hormone (LH) and follicle stimulating hormone (FSH) from the pituitary as well as secondary to direct effects on the ovaries and testes, and such hypogonadism may be associated with bone loss. Hormone Replacement therapy (combined estrogen and progestin therapy) has been effective in increasing bone mass density (BMD) in postmenopausal women with asthma or rheumatoid arthritis who were receiving corticosteroid therapy. Although the efficacy of raloxifene for the prevention or treatment of corticosteroid-induced bone loss remains to be established, some experts (e.g., the American College of

Rheumatology) currently state that raloxifene theoretically should be effective in preventing such bone loss and therefore can be offered to selected postmenopausal corticosteroid-treated women who refuse HRT therapy or other antiresorptive agents (e.g., bisphosphonates, calcitonin) or in whom such therapies are contraindicated.

Reduction in the incidence of breast cancer:

Raloxifene is used to reduce the incidence of invasive breast cancer in postmenopausal women with osteoporosis and in postmenopausal women at high risk of developing invasive breast cancer. Raloxifene should not be used to reduce the risk of breast cancer in premenopausal women.

Raloxifene is not indicated for the treatment of breast cancer or to reduce the risk of recurrence of breast cancer. Raloxifene is not indicated for reduction in the risk of noninvasive breast cancer.

MECHANISM OF ACTION

Raloxifene is a selective estrogen receptor modulator (SERM) with mixed estrogen agonist or antagonist (antiestrogen) activity in specific tissues. Raloxifene exhibits estrogen agonist activity on bone and circulating lipoproteins, but estrogen antagonist activity on breast and uterine tissues. Estrogens have an important role in the reproductive, skeletal, cardiovascular, and central nervous system in women, and act principally by regulating gene expression (ASHP, 2011)

There are three interactive mechanism that explain the pharmacology of SERMs:

- ✓ Differential estrogen-receptor expression in a given target tissue,
- ✓ Differential estrogen-receptor conformation on ligand binding and
- ✓ Differential expression and binding to the estrogen–receptor of coregulator proteins .

There are two main types of estrogen receptors α and β. The α-receptor is mainly an activator and β-receptor inhibitor. The SERMs bind to both receptors, functioning as pure antagonist when binding to β-receptors and as a partial agonist when binding to the α-receptor. The different action of individual SERM depends on coregulatory proteins and their ability to recruit coactivators. Estrogenic activation inhibits osteoclasts and thereby reduces the bone resorption by osteoclasts that restores the balance between bone formation and bone resorption (Helga Hansdottir, 2008).

ADVERSE EFFECT (Helga Hansdottir, 2008)

The increased incidence of venous thromboembolism is the main concern of raloxifene therapy and previous history of venous thromboembolism is contraindication for use of raloxifene.

DRUG INTERACTIONS (ASHP, 2011)

- Colestyramine reduces the absorption and enterohepatic cycling of raloxifene, and they should not be given together.
- Concomitant administration of single doses of raloxifene and warfarin has resulted in a 10% decrease in prothrombin time compared with administration of warfarin alone . In raloxifene-treated women with ospeoporosis, concomitant administration of warfarin did not affect the plasma concentrations of raloxifene. If the drugs are used concomitantly, the patient and prothrombin time should be monitored closely and the dosage of the anticoagulant adusted accordingly

PLURONIC F127

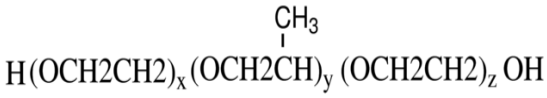
1. Synonyms:

Poloxamer 407, Synperonic PE/F 127 (Badische Anilin – und Soda – Fabrik Corporation, n.d.)

2. Chemical Name:

Poly(ethylene glycol)-block-poly(propylene glycol)-block-poly(ethylene glycol) (BASF Corporation, n.d.)

3. Structure (BASF Corporation, n.d.)**:**



4. Molecular weight:

Molecular weight approximately 12,600 daltons (Diego Chiapetta & Alejandro Sosnik, 2007)

5. Functional category (Raymond Rowe *et al.*, 2009)**:**

Fat emulsifier,
Flavour solubilizer,
Fluorocarbon emulsifier,
Gelling agent,
Spreading agent,
Stabilizing agent,
Suppository base,
Tablet coating,
Tablet excipient and
Wetting agent.

6. Properties:

Melting point: 52-57^{o}C (BASF Corporation, n.d.)

Description: Hydrophilic non-ionic surfactant of the more general class of copolymers known as Poloxamers. Its surfactant properties are dependent on the hydrophilic (EO) / hydrophobic (PO) ratio. High EO content gives better $^{O}/_{W}$ stabilizers (BASF Corporation, n.d.).

Total average number of EO units: 200.5 (Diego Chiapetta & Aljendro Sosnik, 2007)

Total average number of PO units: 65.2 (Diego Chiapetta & Aljendro Sosnik, 2007)

CMC: 0.004 – 0.60%$^{w}/_{w}$ (BASF Corporation, n.d.)

HLB: 22 (BASF Corporation, n.d.)

7. **Applications** (BASF Corporation, n.d.)**:**

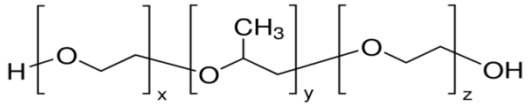
Pluronic F127 was used to coat a siliconized coverslip to hold an egg extract in a study. Pluronic F127 was added to phosphate buffered saline (PBS), to lower unspecific cell and protein adhesion to a PDMS-based microfluidic device. A study reports its use as a release vehicle to transport low-dose perivascular lipopolysaccharide (LPS) on mouse vein grafts. PLGA/pluronic F127 may be used to fabricate nerve guidance conduits (NGCs) for regeneration of peripheral nerve. Fabrication of poly (lactide-co-glycolide) (PLGA) — Pluronic F127 glass composites was reported. Pluronic F-127 was used for fluorescent labeling of blood vessels, astrocytes, and neurons

PLURONIC L121

1. **Chemical Name** (BASF Corporation, n.d.)**:**

Poly(ethylene glycol)-block-poly(propylene glycol)-block-poly(ethylene glycol)

2. **Structure** (BASF Corporation, n.d.)**:**



3. **Molecular weight:**

Molecular weight approximately 4,400 daltons (Diego Chiapetta & Alejandro Sosnik, 2007).

4. **Functional category** (Raymond Rowe *et al.,* 2009)**:**

Fat emulsifier,
Flavour solubilizer,
Fluorocarbon emulsifier,
Gelling agent,
Spreading agent,
Stabilizing agent,
Suppository base,
Tablet coating,
Tablet excipient and

Wetting agent.

5. Properties:

Surface tension: 33 dyn/cm; 25^{o}C, 0.1wt%in H_2O (BASF Corporation, n.d.)

Description: Colorless viscous liquid, surface active material whose properties are dependent on the hydrophilic (EO) / hydrophobic (PO) ratio. High EO content gives better $^{O}/_{W}$ stabilizers (BASF Corporation, n.d.).

Total average number of EO units: 10.0 (Diego Chiapetta & Aljendro Sosnik, 2007)

Total average number of PO units: 68.3 (Diego Chiapetta & Aljendro Sosnik, 2007)

CMC: 0.0004 – 0.005%$^{w}/_{w}$ (BASF Corporation, n.d.)

HLB: 1 (BASF Corporation, n.d.)

6. Applications (BASF Corporation, n.d.)**:**

A non-ionic copolymer surfactant qualified for use in insect cell culture, applications as an antifoaming agent.

The list of drug, excipients used and their manufacturer are shown in Table 2

Table 2: List of Materials Used

S.No.	DRUG/EXCIPIENT	MANUFACTURER
1	Raloxifene Hydrochloride	Orchid Chemicals and Pharmaceuticals Ltd.
2	Pluronic L121	Sigma-Aldrich Chemicals Pvt. Ltd., USA
3	Pluronic F127	Sigma-Aldrich Chemicals Pvt. Ltd., Germany
4	Methanol	Finar limited
5	Tween 80	Scientific Chemicals
6	Potassium dihydrogen ortho phosphate	Thermo Fisher Scientific India Pvt. Ltd.
7	Sodium Hydroxide	Indian Research Products
8	Sucrose	Thermo Fisher Scientific India Pvt. Ltd.
9	Dialysis Membrane 110	Himedia

The list of equipments used in the study and their manufacturer are shown in Table 3.

Table 3: List of Equipments

S.No.	EQUIPMENTS	MANUFACTURER
1	Rotary flash evaporator	Equitron
2	Ultra Sonicator	Lark
3	Electronic balance	Model No – Jewel – 3 Eagle
4	UV-Visible Spectrophotometer	SHIMADZU UV – 1800
5	Magnetic stirrer with hot plate	Eltek Magnetic Stirrers
6	Malvern zeta sizer	Malvern, Germany
7	Super-Resolution and Confocal Microscopy (Zeiss ELYRA PS. 1)	Carl Zeiss Microscopy GmbH, jena, Germany
8	FT-IR Spectrophotometer	Nicolet, India
9	VirTis Advantage Plus Benchtop Freeze Dryer	SP Scientific
10	Refrigerator	Whirlpool

METHODOLOGY

PREPARATION OF PHOSPHATE BUFFER pH 6.8

27.218g of potassium dihydrogen phosphate is dissolved in 1 litre of distilled water to give 0.2M solution, 8g of sodium hydroxide is dissolved in one litre of distilled water to give 0.2M solution. 500ml of 0.2M potassium dihydrogen phosphate and 224ml of 0.2M sodium hydroxide solutions are mixed together and made upto 2 litres with distilled water (MOHFW, 2014).

DRUG EXCIPIENT COMPATIBILITY STUDIES (Gurdeep Chatwal and Sham Anand, 2002)

Infrared spectroscopy can be used to identify a compound and also to investigate the composition of the mixture. Pure drug and Drug-Excipient mixtures were subjected to FT-IR to investigate the Drug-Excipient interactions. The IR spectra of the test samples were obtained by Pressed Pellet Technique using Potassium bromide.

Potassium bromide pellet method

A small amount of finely ground solid sample was intimately mixed with about 100 times of its weight of powdered Potassium bromide. The finely ground mixture was then passed under high pressure in a press (at least 25,000 psig) to form a small pellet (about 1-2 mm thick and 1 cm in diameter). The resulting pellet was placed in the sample cell and the spectra were recorded.

STANDARD CURVE FOR RALOXIFENE HYDROCHLORIDE (RXH)

100mg of Raloxifene Hydrochloride was accurately weighed and dissolved in a small quantity of methanol and made upto 100ml with methanol. From this primary solution, 10ml was pipette out and made up to 100ml with phosphate buffer pH 6.8. From this secondary solution aliquots were taken to produce concentration of 2, 4, 6, 8 and 10 µg/ml. The absorbance of the resulting solution was measured at 285nm in UV- Visible Spectrophotometer (Shimadzu) using phosphate buffer pH 6.8 as blank. The standard curve was plotted taking concentration in X-axis and absorbance in Y-axis (Anand Kumar Kushwaha *et al.*, 2013)

FORMULATION OF RALOXIFENE HYDROCHLORIDE POLYMERIC MICELLES

The RXH loaded mixed pluronic polymeric micelles were prepared by film rehydration method. Accurately weighed quantities of RXH, Pluronic L121 and Pluronic F127 were dissolved in sufficient quantity of methanol to give a clear solution. The resulting solution is poured into a 1000ml rotary flask and evaporated under vacuum at room temperature for 2 hours to yield the L121/F127 matrix film. Distilled water was added to the matrix film to obtain $1\%^{w}/_{w}$ fraction of Pluronic F127 and Pluronic L121; and 1%w/w fraction of either polymer. The round bottom flask was gently rotated for 1 hour at 30 – 40^{o}C. The resulting polymeric L121/F127 micelle dispersions were allowed to equilibrate at room temperature for 24 hours followed by sonication with ultrasonicator for 2 mins and then the optimized micellar dispersion is concentrated with the help of lyophilizer using cryoprotective agent, sucrose ($2.5\%^{w}/_{w}$) (Ivan Pepic *et al.,* 2014).

Table 4: Formulation Code of L121/F127 micelle dispersions

FORMULATION CODE	DRUG (RALOXIFENE HYDROCHLORIDE) (mg)	PLURONIC L121 (mg)	PLURONIC F127 (mg)	WATER added to produce
F-1	60	170	500	$1\%^{w}/_{w}$ fraction of F127
F-2	60	250	500	$1\%^{w}/_{w}$ fraction of F127
F-3	60	500	170	$1\%^{w}/_{w}$ fraction of L121
F-4	60	500	250	$1\%^{w}/_{w}$ fraction of L121
F-5	60	300	300	$1\%^{w}/_{w}$ fraction of L121 and F127
F-6	60	500	500	$1\%^{w}/_{w}$ fraction of L121 and F127

CHARACTERIZATION OF POLYMERIC L121/F127 MICELLE DISPERSIONS

ENTRAPMENT EFFICIENCY (Liyan Zhao et al., 2012)

The concentration of RXH in the micelles were determined with UV-Vis Spectrophotometer (UV-1800, Shimadzu) at 285nm. The micelles were lysed using 15ml of methanol and the concentration was determined using UV-Vis Spectrophotometer after suitable dilution. The percentage of drug entrapment in micelle dispersions was calculated using the following formula,

$$EE\% = \frac{\text{Weight of the drug in the micelles}}{\text{Weight of the feeding polymer and drug}} \times 100$$

***IN-VITRO* DIFFUSION STUDY**

In-vitro release studies werer performed using pH 6.8 phosphate buffer containing $0.5\%^{v}/_{v}$ polysorbate 80 by dialysis bag method using dialysis membrane having molecular weight cut off 12,000- 14,000 daltons. Micelle dispersions was filled into a dialysis membrane bag and tied at both the ends and placed in a 1000ml beaker containing 900ml of diffusion medium; temperature and speed were maintained at $37^{o}C$ and 100rpm, respectively using magnetic stirrer. 5 ml of samples were collected at a predetermined time and replenished with the same volume of fresh buffer to maintain the sink condition. Samples were analyzed at 285nm UV spectrophotometrically (Anand Kumar Kushwaha *et al.,* 2013).

KINETICS OF DRUG RELEASE (Brahmankar and Sunil Jaiswal, 2009)

Various models were tested for explaining the kinetics of drug release. To analyze the mechanism of the drug release rate kinetics of the dosage form, the obtained data were plotted in various kinetic models (Zero-order, First order, Higuchi, Hixson-Crowell release model and Korsmeyer-Peppas release model).

1. Zero order equation

The zero order release can be obtained by plotting cumulative % percentage drug release versus time. It is ideal for the formulation to have release profile of zero order to achieve pharmacological prolonged action.

$$\mathbf{C = K_0 t}$$

Where, K_0 = Zero order constant

t = Time in hours

2. First order equation

The graph was plotted as log % cumulative drug remaining Vs time in hours.

$$\mathbf{Log\ C = log\ C_0 - Kt/2.303}$$

Where, C_0 = Initial concentration of drug

K = First order

t = Time in hours

3. Higuchi kinetics

The graph was plotted with % cumulative drug released vs. square root of time

$$\mathbf{Q = Kt^{½}}$$

Where, K= constant reflecting design variable system (differential rate constant)

t = Time in hours

4. Hixon and Crowell erosion equation

To evaluate the drug release with changes in the surface area and the diameter of particles, the data were plotted using the Hixon and Crowell rate equation. The graph was plotted by cube root of % drug remaining vs. time in hours.

$$\mathbf{Q_0^{1/3} - Q_t^{1/3} = K_{HC}Xt}$$

Where, Q_t = amount of drug released in time t.

Q_0 = Initial Amount of drug

K_{HC}= Rate constant for Hixon Crowell equation

5. Korsmeyer-Peppas equation

To evaluate the mechanism of drug release, it was further plotted in Peppas equation as log cumulative % of drug released Vs.log time.

$$\mathbf{M_t/M_\alpha = Kt^n}$$

Where, $\mathbf{M_t/M_\alpha}$ **=** Fraction of drug released at time t

t = Release time

K=Kinetics constant (Incorporating structural and geometric characteristics of the formulation)

n = Diffusional exponent indicative of the mechanism of drug release.

Table 5: Diffusion mechanism is given by the slope (n) value,

n value	Mechanism
0.45	Fickian diffusion
0.45 < n < 0.89	Anomalous (non- Fickian) diffusion
0.89	Case II transport
n > 0.89	Super case II transport

The models were used to analyze the release of pharmaceutical polymeric dosage forms when the release mechanism was not known or more than one type of release was involved. The r^2 and K values were calculated for the linear curve obtained by regression analysis of the above plots

PARTICLE SIZE ANALYSIS

The micelle dispersion was diluted, filled in a cuvette using suitable blank and the average size and polydispersity index (PDI) was determined using Malvern zeta sizer (Samyuktha Rani & Vedha Hari, 2011).

POLYDISPERSITY INDEX

The term polydispersity is used to describe the degree of "non –uniformity" of a distribution (material talks). The Polydispersity index as a measure of the width of molecular weight distribution (MWD) (Ulf Nobbman, 2014).

Table 6: Polydispersity Index (Ryan Shaw, n.d.)

POLYDISPERSITY INDEX VALUE (PDI)	COMMENTS
<0.05	Monodisperse sample.
<0.08	Nearly monodisperse sample.
0.08 to 0.7	Mid-range value of PDI. It is the range over which the distributions algorithms best operate.
>0.7	Indicates a very broad distribution of particle size

ZETA POTENTIAL ANALYSIS (Ye Jin et al., 2013)

Zeta potential analysis was used to measure the stability of micelle dispersions by studying its colloidal property. Aggregation is attributed to the shielding of the micelle surface charge by ions in the solution and thereby reducing the electrostatic repulsion. Micelle surface charge can be estimated by measurement of particle electrophoretic mobility and is expressed as the zeta potential. The study was conducted using Malvern Zeta Analyzer

SOLUBILITY STUDIES

Solubility study of Raloxifene Hydrochloride

An excess amount of drug is added to the distilled water and then left until equilibrium is established for 72 hrs at room temperature with occasional stirring. Then filtered and the filterate is suitably diluted with phosphate buffer pH 6.8 and analyzed using UV-Visible spectrophotometer at 285nm. The concentration is considered is considered the saturation or equilibrium solubility of drug (Ramy Elsergany, 2014).

Solubility study of optimized formulation

The optimized formulation is filtered and the filterate is suitably diluted with phosphate buffer pH 6.8 and analyzed using UV-Visibe spectrophotometer at 285 nm (Carlota Rangel – Yagui *et al.,* 2013).

SUPER-RESOLUTION AND CONFOCAL MICROSCOPY (Samuel G.L., 2017)

The morphology of micelle dispersions were measured by super resolution and confocal microscopy. A small amount of the sample is placed in the mounting medium (Prolong Gold) on the slide. Cover slip is placed over the sample with a spacer and with the help of nail polish it is sealed and let dried. After which, using zen software, high resolution 2D and 3D fluorescence images were established.

LYOPHILIZATION OF THE L121/F127 MICELLES (Ivan Pepic et al., 2014)

The micelles were prepared using the film rehydration method with ultrasonication. The preparation which yielded best results were selected for the freeze-drying process.

2.5%$^{w}/_{w}$ of sucrose is added as cryoprotective agent to micelle dispersions. After which, aliquots of the L121/F127 micelle dispersions are transferred to freeze-drying flask. The samples were frozen by rotating the flask in a bath called a shell freezer which is cooled by mechanical refrigeration at -70^{o}C for 3 hours. After freezing, the micelle dispersions were uncapped and transferred into a -53^{o}C pre-equilibrated chamber of an VirTis Advantage Plus Benchtop Freeze Dryer (SP Scientific) which was depressurized using a vacuum pump. The temperature was maintained at -53^{o}C during the 24h of primary drying and the concentrated dispersion was removed after reestablishing ambient pressure, the vials were capped and stored at 2 to 8^{o}C until further use.

DRUG EXCIPIENT COMPATIBILITY STUDIES

The possible interaction between the drug and the excipients used in the formulation was studied by FTIR spectroscopy. The results are given in the below

FTIR SPECTRA OF DRUG (RALOXIFENE HYDROCHLORIDE)

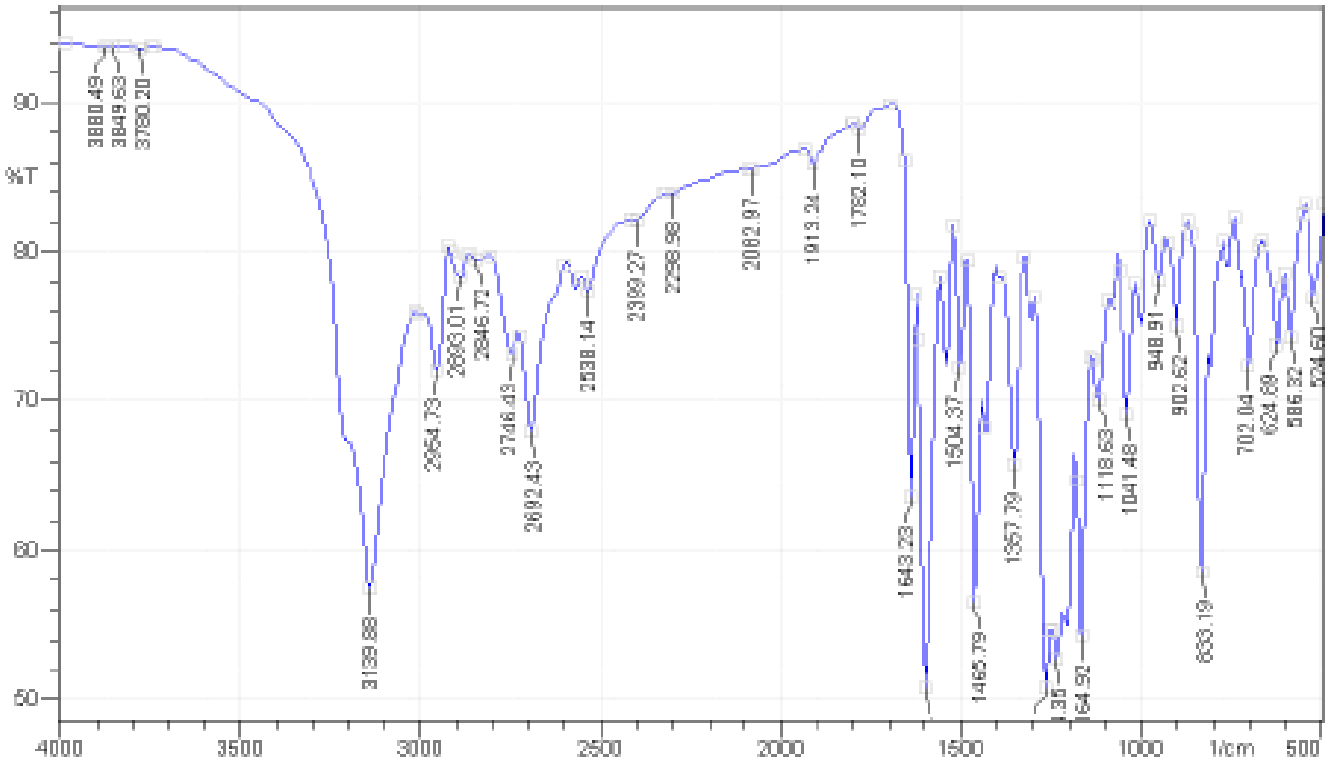


Fig. 5: FT–IR spectra of Raloxifene Hydrochloride

Table 7: Interpretation

S.No.	WAVENUMBER (cm^{-1})	CHARACTERISTICS
1	2954.73	C-H stretching
2	1643.23	C=C stretching
3	1465.79	O-H bending
4	948.91	C-O stretching
5	833.19	C-C stretching
6	702.04	N-H rocking

FTIR SPECTRA OF RALOXIFENE HYDROCHLORIDE WITH PLURONIC F127

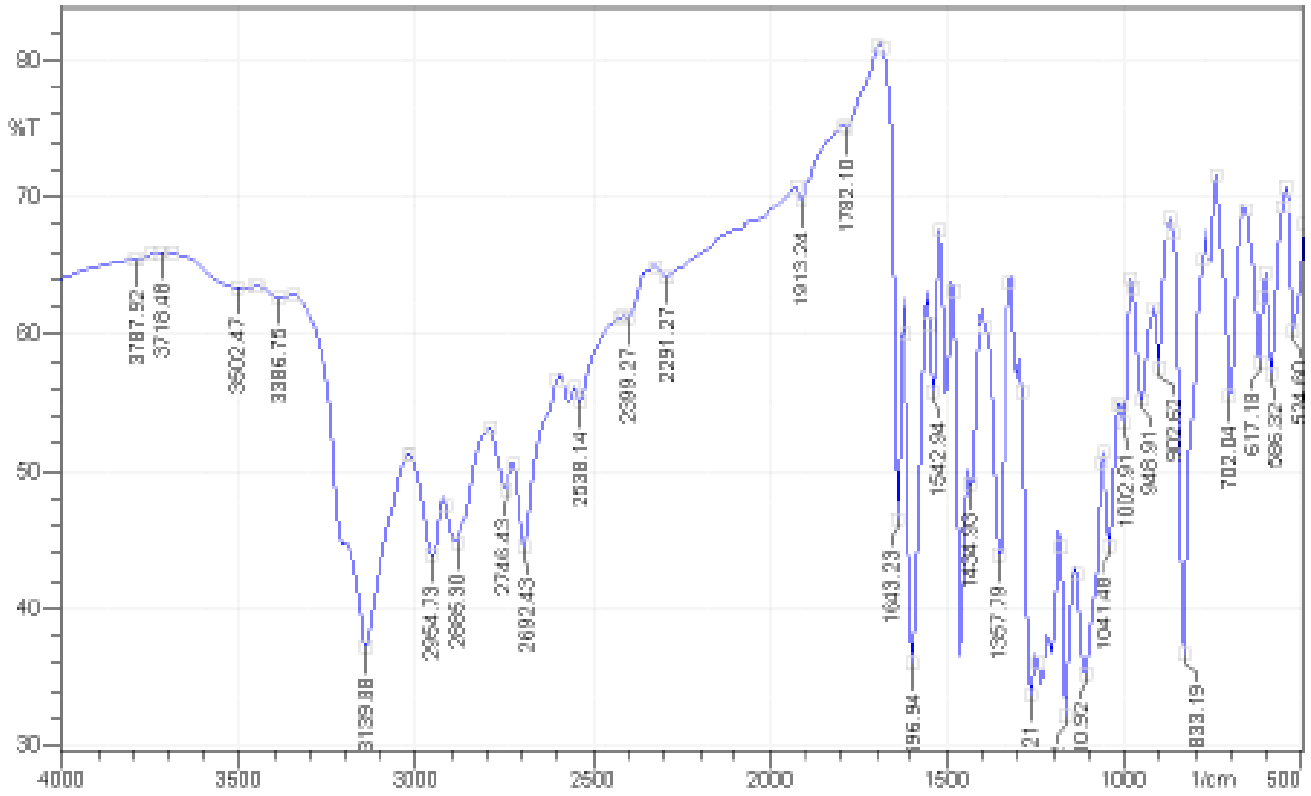


Fig. 6: FT–IR spectra of Raloxifene Hydrochloride with Pluronic F127

Table 8: Interpretation

S.No.	WAVENUMBER (cm^{-1})	CHARACTERISTICS
1	2954.73	C-H stretching
2	1643.23	C=C stretching
3	1434.93	O-H bending
4	948.91	C-O stretching
5	833.19	C-C stretching
6	702.04	N-H rocking

The peaks observed in the FTIR spectrum showed no shift and no disappearance of characteristic peaks of drug. This suggests that there was no interaction between the drug and Pluronic F127 (Gurdeep Chatwal and Sham Anand, 2002).

FTIR SPECTRA OF RALOXIFENE HYDROCHLORIDE WITH PLURONIC L121

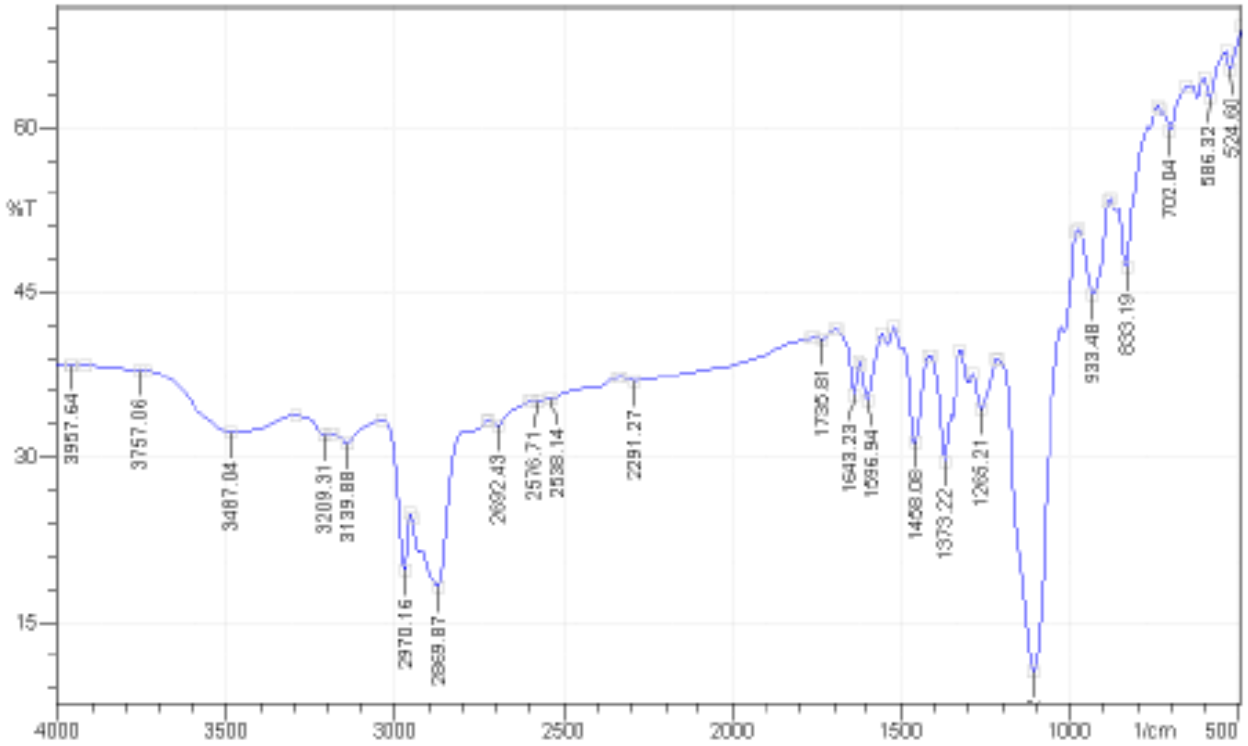


Fig. 7: FT–IR spectra of Raloxifene Hydrochloride with Pluronic L121

Table 9: Interpretation

S.No.	WAVENUMBER (cm^{-1})	CHARACTERISTICS
1	2970.16	C-H stretching
2	1643.23	C=C stretching
3	1458.08	O-H bending
4	933.48	C-O stretching
5	833.19	C-C stretching
6	702.04	N-H rocking

The peaks observed in the FTIR spectrum showed no shift and no disappearance of characteristic peaks of drug. This suggests that there was no interaction between the drug and Pluronic L121 (Gurdeep Chatwal and Sham Anand, 2002).

FTIR SPECTRA OF RALOXIFENE HYDROCHLORIDE WITH PLURONIC F127 AND L121

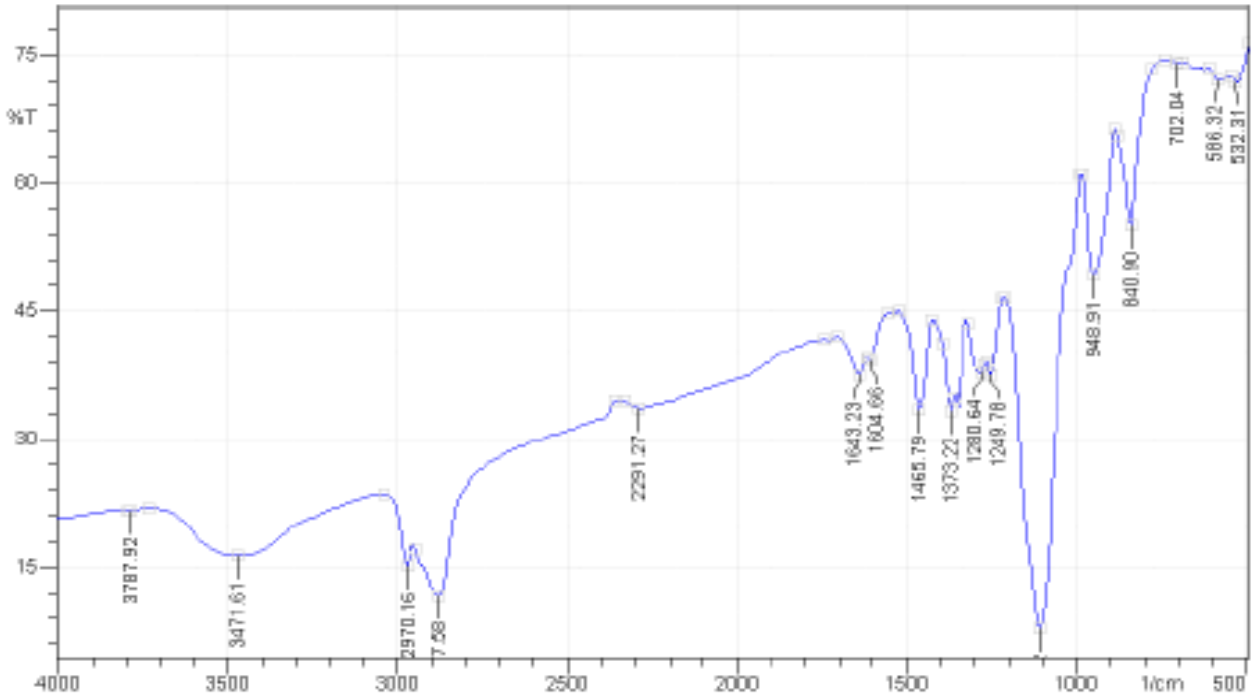


Fig. 8: FT–IR spectra of Raloxifene Hydrochloride with Pluronic F127 and L121

Table 10: Interpretation

S.No.	WAVENUMBER (cm^{-1})	CHARACTERISTICS
1	2970.16	C-H stretching
2	1643.23	C=C stretching
3	1465.79	O-H bending
4	948.91	C-O stretching
5	840.90	C-C stretching
6	702.04	N-H rocking

The peaks observed in the FTIR spectrum showed no shift and no disappearance of characteristic peaks of drug. This suggests that there was no interaction between the drug and drug loaded polymeric micelles (Gurdeep Chatwal and Sham Anand, 2002).

STANDARD CURVE OF RALOXIFENE HYDROCHLORIDE

The UV spectrophotometric method was used to analyse Raloxifene Hydrochloride. The absorbance of the drug in phosphate buffered saline pH6.8 was measured at a wavelength of 285nm. The results are given in the following table and figure

Table 11: Data for Calibration Curve of Raloxifene Hydrochloride

S. No.	Concentration (µg/ml)	Absorbance at 285 nm
1	2	0.104±0.00294
2	4	0.233±0.00169
3	6	0.341±0.01228
4	8	0.449±0.01806
5	10	0.563±0.01673

*Mean± SD (n=3)

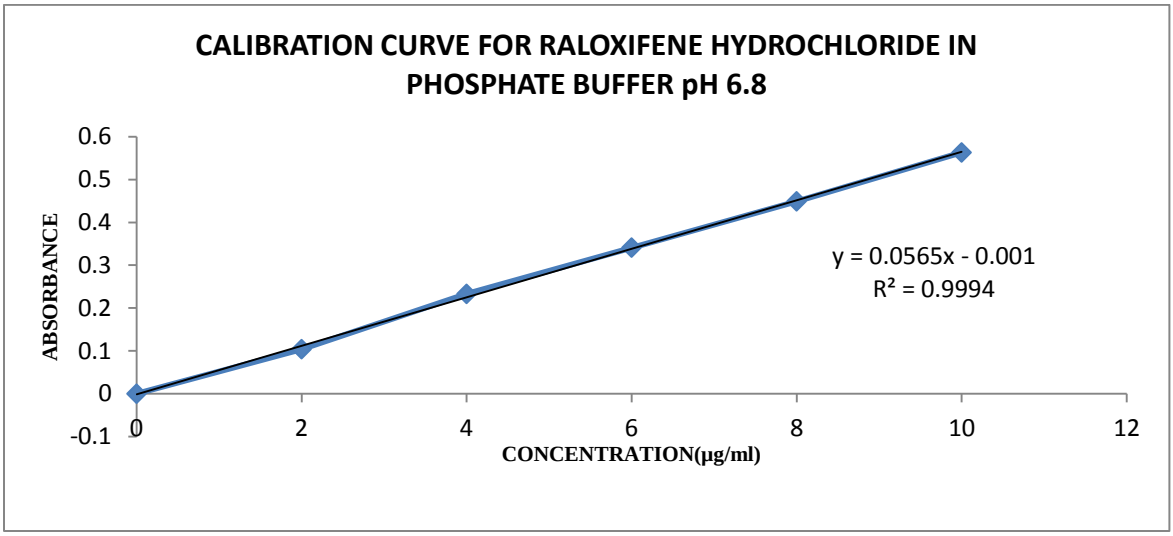


Fig. 9: Standard Curve of Raloxifene Hydrochloride in PBS pH 6.8

The standard curve of Raloxifene Hydrochloride in PBS pH6.8 was linear in 2 to 10µg/ml concentrations , starting from origin. The curve obeys Beer Lambert's law (Gurdeep Chatwal & Sham Anand, 2002).

CHARACTERIZATION OF MICELLAR DISPERSIONS

- **ENTRAPMENT EFFICIENCY**

Table 12 : Entrapment efficiency of Raloxifene Hydrochloride loaded micellar dispersions

FORMULATION CODE	% ENTRAPMENT EFFICIENCY (% w/w)
F-1	94.75
F-2	95.00
F-3	94.16
F-4	92.50
F-5	95.50
F-6	95.83

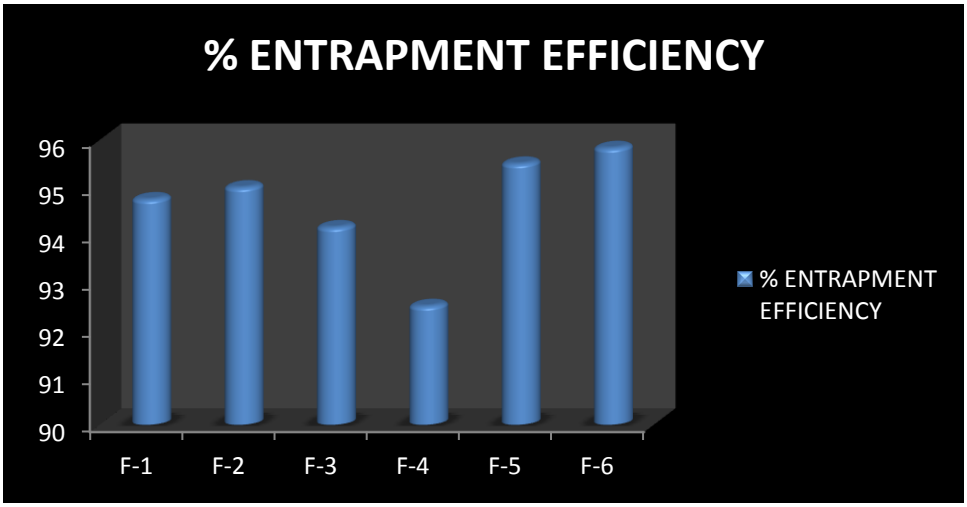


Fig. 10: Entrapment efficiency of Raloxifene Hydrochloride loaded micellar dispersions

The entrapment efficiency of all the formulations was between 92 to 96%. It was found to be 94.75, 95, 94.16, 92.5, 95.5 and 95.83% for F-1, F-2, F-3, F-4, F-5 and F-6 respectively.

- ***IN-VITRO* DIFFUSION STUDY**

The *in-vitro* release study of Raloxifene Hydrochloride micellar dispersions were performed using pH 6.8 phosphate buffer containing 0.5%$^{v}/_{v}$ polysorbate 80 by dialysis bag method using dialysis membrane having molecular weight cut off 12,000 – 14000 daltons.

Table 13: *In-vitro* release of Raloxifene Hydrochloride loaded micellar dispersions

Time (in hours)	F-1	F-2	F-3	F-4	F-5	F-6
0.5	14.58	9.27	0.78	2.13	5.58	1.59
1	17.60	12.77	2.64	3.19	9.84	3.18
1.5	19.28	15.24	4.009	5.33	11.75	5.60
2	21.25	16.10	4.54	6.14	13.41	9.32
3	22.69	17.27	5.37	6.72	14.29	9.37
4	24.67	18.41	5.67	6.75	14.61	9.70
5	26.66	19.32	5.70	7.06	15.77	10.02
23	54.38	33.74	16.86	21.41	29.65	22.55
24	57.59	34.19	17.5	22.07	30.32	22.95
25	59.76	35.43	17.59	22.19	31.30	23.58
26	61.67	36.43	18.47	22.85	32.25	23.71
27	62.55	37.88	18.84	23.24	33.50	24.38
28	63.93	38.42	19.21	23.44	34.46	24.78
29	66.41	39.43	19.31	23.74	35.73	24.91
47	77.92	59.26	29.77	-	48.91	37.50
48	81.51	60.39	30.71	-	49.71	37.97
49	85.40	61.76	31.41	-	49.97	38.17
50	89.30	63.14	31.58	-	51.02	38.64
51	92.14	64.29	32.02	-	52.1	39.12
52	-	65.95	32.46	-	53.15	39.32
53	-	67.10	32.90	-	54.27	39.8
71	-	82.81	42.61	-	68.84	56.99
72	-	84.84	42.84	-	69.74	57.29
73	-	87.41	43.33	-	70.64	58.10
74	-	89.19	44.07	-	71.79	58.41
75	-	90.46	44.30	-	72.97	59.25
76	-	91.47	45.07	-	74.13	59.56
77	-	92.46	45.84	-	74.78	60.41
95	-	-	-	62.59	86.84	70.53
96	-	-	-	63.74	87.82	70.90
97	-	-	-	64.36	89.06	71.53
98	-	-	-	64.71	90.05	72.41
99	-	-	-	65.6	-	72.78
100	-	-	-	66.19	-	73.69
101	-	-	-	66.54	-	74.06
119	-	-	65.16	77.51	-	89.04

120	-	-	67.12	78.19	-	89.50
121	-	-	67.47	78.87	-	90.46
122	-	-	68.06	79.56	-	91.46
123	-	-	68.41	79.97	-	-
124	-	-	69.03	80.90	-	-
125	-	-	69.66	81.59	-	-
143	-	-	78.50	88.64	-	-
144	-	-	78.91	89.64	-	-
145	-	-	79.58	90.64	-	-
146	-	-	80.52	91.10	-	-
147	-	-	81.17	-	-	-
148	-	-	81.85	-	-	-
149	-	-	82.26	-	-	-
167	-	-	89.03	-	-	-
168	-	-	89.74	-	-	-

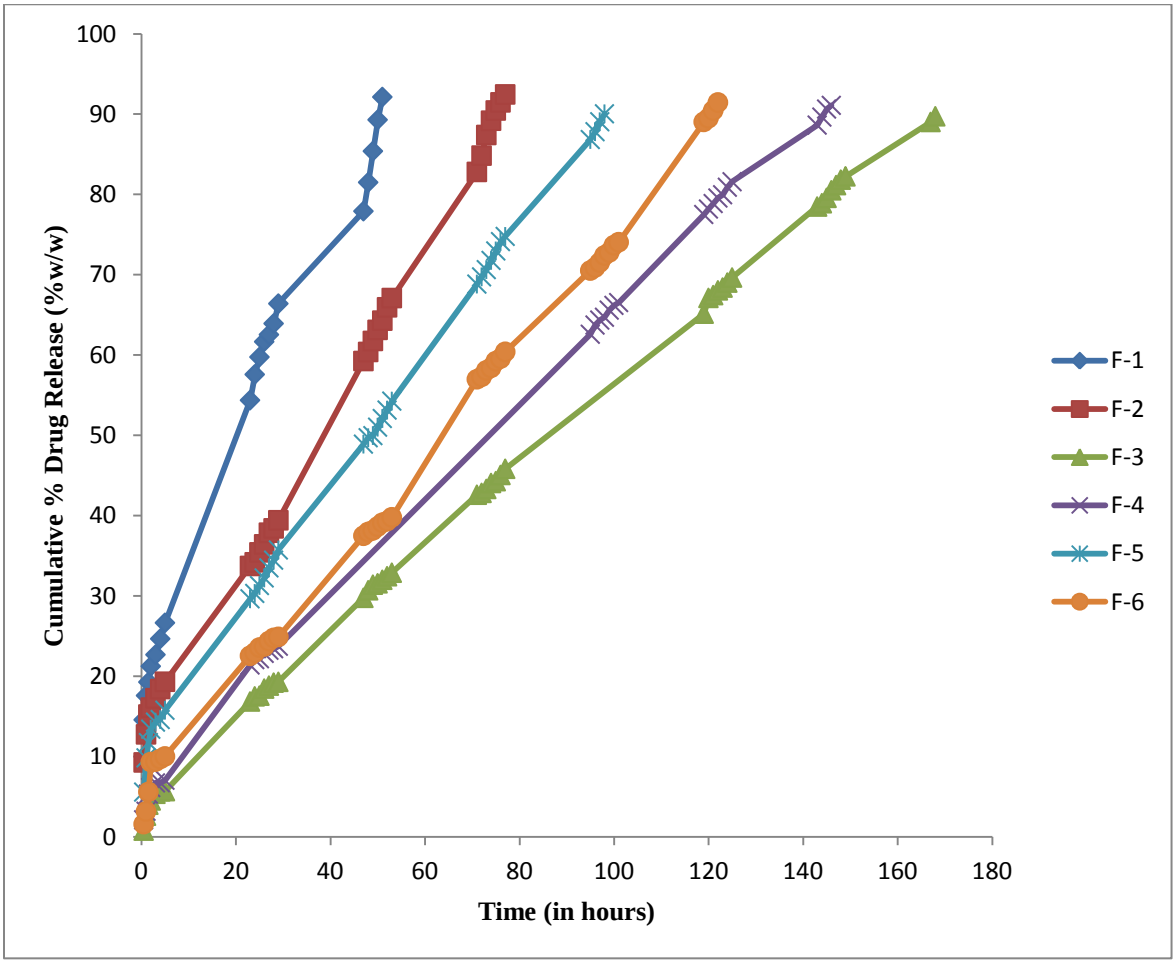


Fig. 11: *In-vitro* release of Raloxifene Hydrochloride loaded micellar dispersions

The cumulative % drug release at 29 hours is 66.41, 39.43, 19.31, 23.74, 35.73 and 24.91% for F-1, F-2, F-3, F-4, F-5 and F-6 respectively.

Increase in concentration of hydrophilic polymer resulted in release of drug (92.14 and 92.46%) at 51 and 77 hours respectively for F-1 and F-2.

Increase in the concentration of hydrophilic polymer (pluronic F127) increase the PEO units leading to more hydrophilic channel and faster release of drug (Zhang Wei *et al.,* 2009)

Increase in the hydrophobic polymer (pluronic L121) concentration, sustains the release of drug (Jaber Emami *et al.*, 2015). For eg: increase in pluronic L121 concentration shows release of drug (89.74 and 91.10%) at 168 and 146 hours respectively for F-3 and F-4.

The micellar dispersions with same ratio of L121/F127 shows release of drug (90.05 and 91.46%) at 98 and 122 hours respectively for F-5 and F-6.

The hydrophobic core is essential and hydrophilic corona prevent recognition by the RES (Bhagwat and Vaidhya, 2013) and therefore preliminary elimination of the micelles from the blood stream, this may result in prolonged circulation.

Though increase in pluronic L121 concentration shows sustained release, the use of F127 is essential because it has longer PEO units. Thus using the same ratio of both L121 and F127 may result in prolonged circulation as micelles with blocks made of poly (ethylene oxide) sterically stabilized (stealth) and may undergo less opsonization and uptake by the macrophages of RES, allowing the micelles to circulate longer in blood (Diego Chiappetta & Alejandro Sosnik, 2007).

KINETICS OF DRUG RELEASE

The *in-vitro* release data was applied to various kinetic models to predict the mechanism of drug release of optimized formulation

Table 14: Release Kinetics of Formulation F-6

Time (hrs)	Log time (hrs)	Square root of time (hrs)	Cumulative % drug release	Cumulative % drug remaining	Log Cumulative % drug release	Log Cumulative % drug remaining	Cube root of Cumulative % drug remaining
0	∞	0	0	100	∞	2	4.64
0.5	-0.301	0.7071	1.59	98.41	0.2013	1.993	4.61
1	0	1	3.18	96.82	0.5024	1.9859	4.59
1.5	0.176	1.2247	5.6	94.4	0.7481	1.9749	4.55
2	0.301	1.414	9.32	90.68	0.9694	1.9575	4.49
3	0.4771	1.732	9.37	90.63	0.9717	1.9572	4.49
4	0.602	2	9.7	90.3	0.9867	1.9556	4.48
5	0.6989	2.236	10.02	89.98	1.0008	1.9541	4.48
23	1.3617	4.7958	22.55	77.45	1.3531	1.889	4.26
24	1.3802	4.8989	22.95	77.05	1.3607	1.8867	4.25
25	1.3979	5	23.58	76.42	1.3725	1.8832	4.24
26	1.4149	5.099	23.71	76.29	1.3749	1.8824	4.24
27	1.4313	5.1961	24.38	75.62	1.387	1.8786	4.23
28	1.4471	5.2915	24.78	75.22	1.3941	1.8763	4.22
29	1.4623	5.3851	24.91	75.09	1.3963	1.8755	4.22
47	1.672	6.8556	37.5	62.5	1.574	1.7958	3.97
48	1.681	6.9282	37.97	62.03	1.5794	1.7926	3.96
49	1.69	7	38.17	61.83	1.5817	1.7911	3.95
50	1.698	7.071	38.64	61.36	1.587	1.7878	3.94
51	1.7075	7.1414	39.12	60.88	1.5923	1.7844	3.93
52	1.716	7.2111	39.32	60.68	1.5946	1.783	3.93
53	1.7242	7.2801	39.8	60.2	1.5998	1.7795	3.92
71	1.8512	8.4261	56.99	43.01	1.7557	1.6335	3.5
72	1.8573	8.4852	57.29	42.71	1.758	1.6305	3.49
73	1.8633	8.544	58.1	41.9	1.7642	1.6222	3.47
74	1.8692	8.6023	58.41	41.59	1.7665	1.6189	3.46
75	1.875	8.6602	59.25	40.75	1.7727	1.6101	3.44
76	1.8808	8.7177	59.56	40.44	1.7749	1.6068	3.43
77	1.8864	8.7749	60.41	39.59	1.7811	1.5975	3.41
95	1.9777	9.7467	70.53	29.47	1.8483	1.4693	3.09
96	1.9822	9.7979	70.9	29.1	1.8506	1.4638	3.08
97	1.9867	9.8488	71.53	28.47	1.8545	1.4543	3.05
98	1.9912	9.8994	72.41	27.59	1.8598	1.4407	3.02
99	1.9956	9.9498	72.78	27.22	1.862	1.4348	3.01
100	2	10	73.69	26.31	1.8674	1.4201	2.97
101	2.0043	10.0498	74.06	25.94	1.8696	1.4139	2.96
119	2.0755	10.9087	89.04	10.96	1.9495	1.0398	2.22
120	2.0791	10.9544	89.5	10.5	1.9518	1.0211	2.19
121	2.0827	11	90.46	9.54	1.9564	0.9795	2.12
122	2.0863	11.0453	91.46	8.54	1.9612	0.9314	2.04

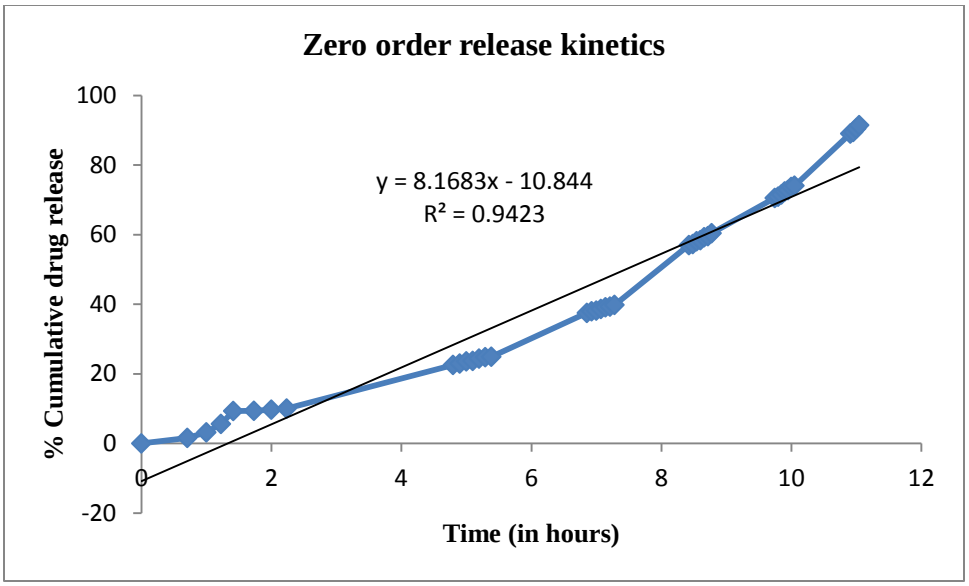


Fig. 12 : Zero-Order Release Kinetics of F-6

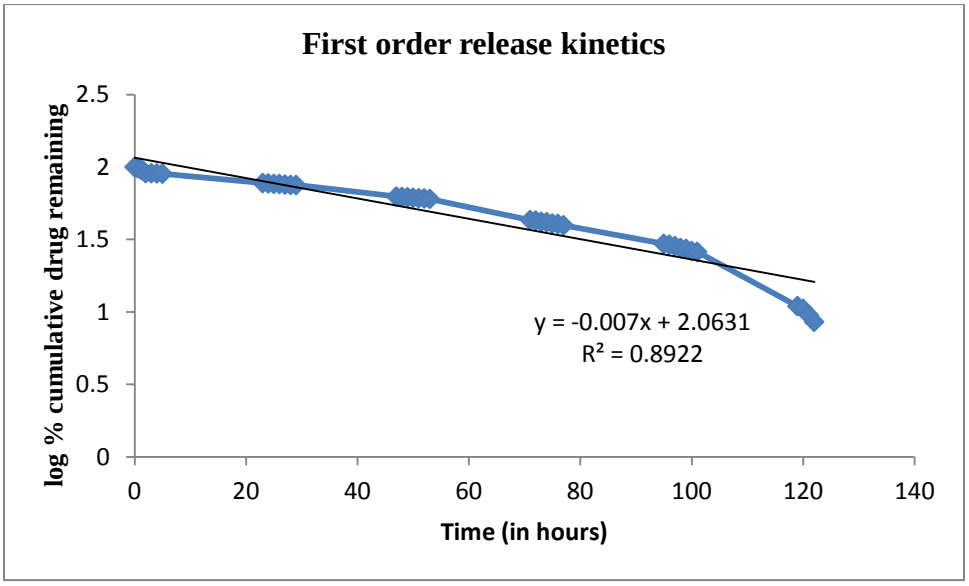


Fig. 13: First Order Release Kinetics of F-6

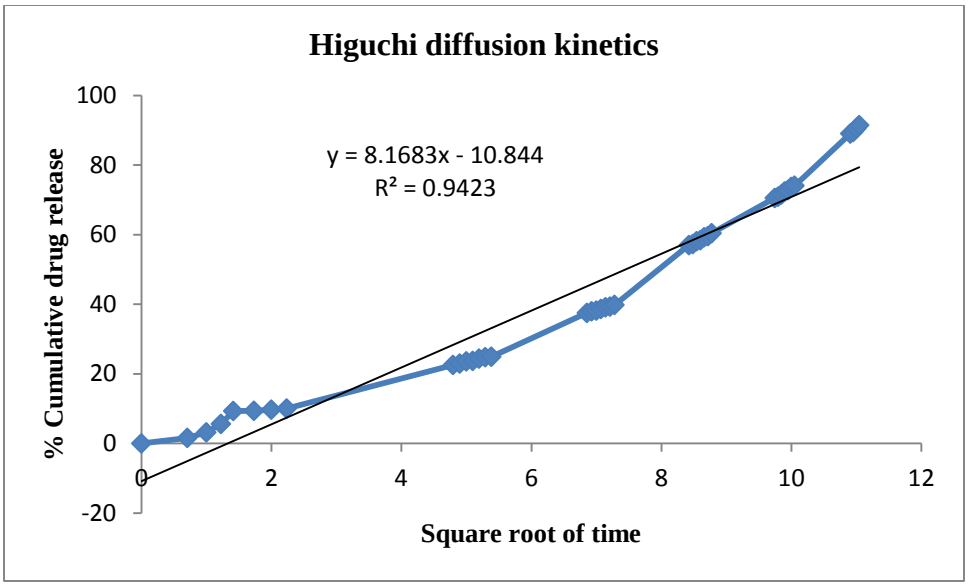


Fig. 14 : Higuchi diffusion kinetics of F-6

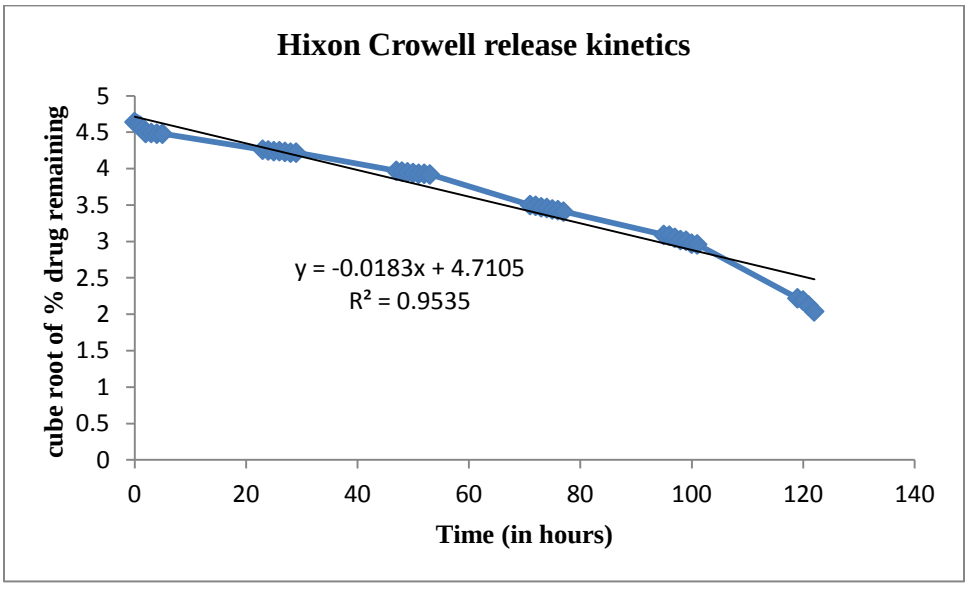


Fig. 15: Hixon Crowell Model Kinetics of F-6

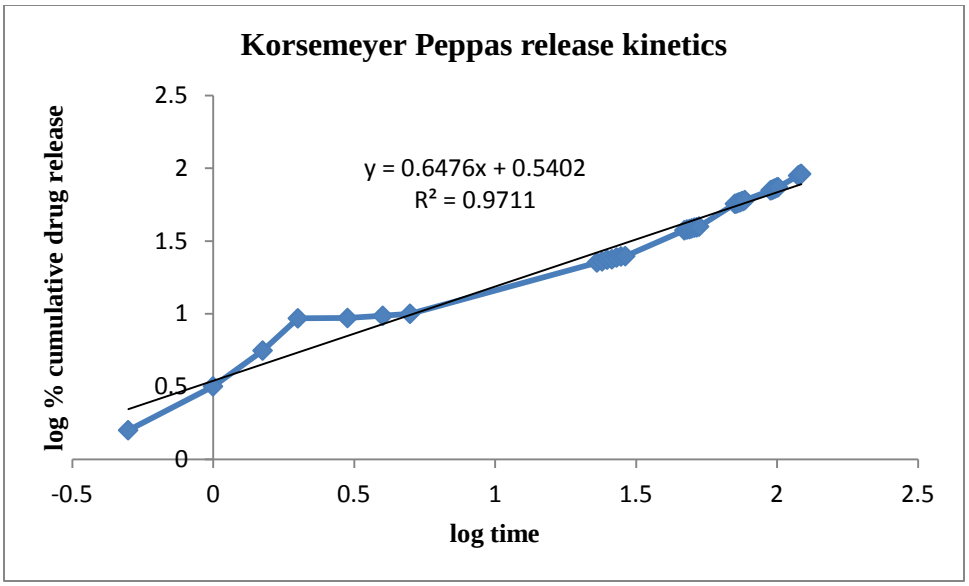


Fig. 16: Korsemeyer- Peppas Model Kinetics of F-6

The optimized formulation (F-6) follows zero order kinetics in which the regression was 0.9423.

The ‘n’value of Korsemeyer-Peppas equation was found to be 0.6476. From this it was concluded that the drug release follows non-fickian diffusion (Brahmankar & Sunil Jaiswal, 2009).

MAVERN PARTICLE SIZE ANALYSIS OF F-2

Size Statistics Report by Volume

Malvern

Sample Details

Sample Name: LF3
File Name: PM3.dts
SOP Name: mansettings.nano
Measurement Date and Time: Tuesday, Mar 14, 2017 5:51:32 PM

Z-Average (nm):	25.37899	**Derived Count Rate (kcps):**	2917.8332814...
Standard Deviation (nm):	0	**Standard Deviation (kc...**	0
%Std Deviation:	0	**%Std Deviation:**	0
Variance:	0	**Variance:**	0

Size d.nm	Mean Volume %	Std Dev Volume %	Size d.nm	Mean Volume %	Std Dev Volume %	Size d.nm	Mean Volume %	Std Dev Volume %	Size d.nm	Mean Volume %	Std Dev Volume %
0.4000	0.0		5.615	0.0		78.82	0.0		1106	0.0	
0.4632	0.0		6.503	0.0		91.28	0.0		1281	0.0	
0.5365	0.0		7.531	0.0		105.7	0.0		1484	0.0	
0.6213	0.0		8.721	0.0		122.4	0.0		1718	0.0	
0.7195	0.0		10.10	0.0		141.8	0.0		1990	0.0	
0.8332	0.0		11.70	0.5		164.2	0.0		2305	0.0	
0.9649	0.0		13.54	2.4		190.1	0.0		2669	0.0	
1.117	0.0		15.69	5.8		220.2	0.0		3091	0.0	
1.294	0.0		18.17	11.6		255.0	0.0		3580	0.0	
1.499	0.0		21.04	13.3		295.3	0.0		4145	0.0	
1.736	0.0		24.36	17.7		342.0	0.0		4801	0.0	
2.010	0.0		28.21	20.9		396.1	0.0		5560	0.0	
2.328	0.0		32.67	11.7		458.7	0.0		6439	0.0	
2.696	0.0		37.84	6.1		531.2	0.0		7456	0.0	
3.122	0.0		43.82	4.5		615.1	0.0		8635	0.0	
3.615	0.0		50.75	3.8		712.4	0.0		1.000e4	0.0	
4.187	0.0		58.77	1.3		825.0	0.0				
4.849	0.0		68.06	0.6		955.4	0.0				

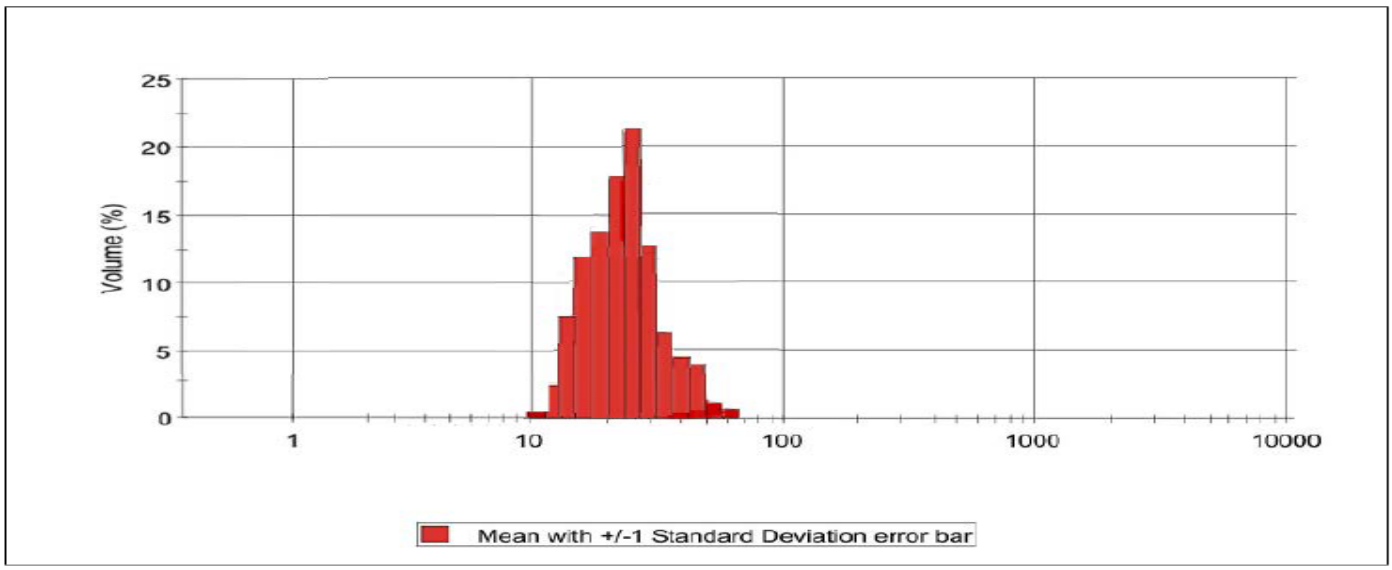


Fig. 17: Statistics graph of F-2 formulation

Size Distribution Report by Intensity

v2.1

Malvern

Sample Details

Sample Name:	LF3		
SOP Name:	mansettings.nano		
General Notes:			
File Name:	PM3.dts	**Dispersant Name:**	Water
Record Number:	3	**Dispersant RI:**	1.330
Material RI:	1.35	**Viscosity (cP):**	0.8872
Material Absorbtion:	0.10	**Measurement Date and Time:**	Tuesday, Mar 14, 2017 5:5...

System

Temperature (°C):	25.0	**Duration Used (s):**	60
Count Rate (kcps):	312.7	**Measurement Position (mm):**	4.25
Cell Description:	Disposable sizing cuvette	**Attenuator:**	4

Results

			Size (d.n...	% Intensity	Width (d.n...
Z-Average (d.nm):	25.37	**Peak 1:**	20.80	77.5	9.428
PdI:	0.513	**Peak 2:**	187.4	19.8	91.51
Intercept:	0.929	**Peak 3:**	5111	2.7	542.6
Result quality :	Good				

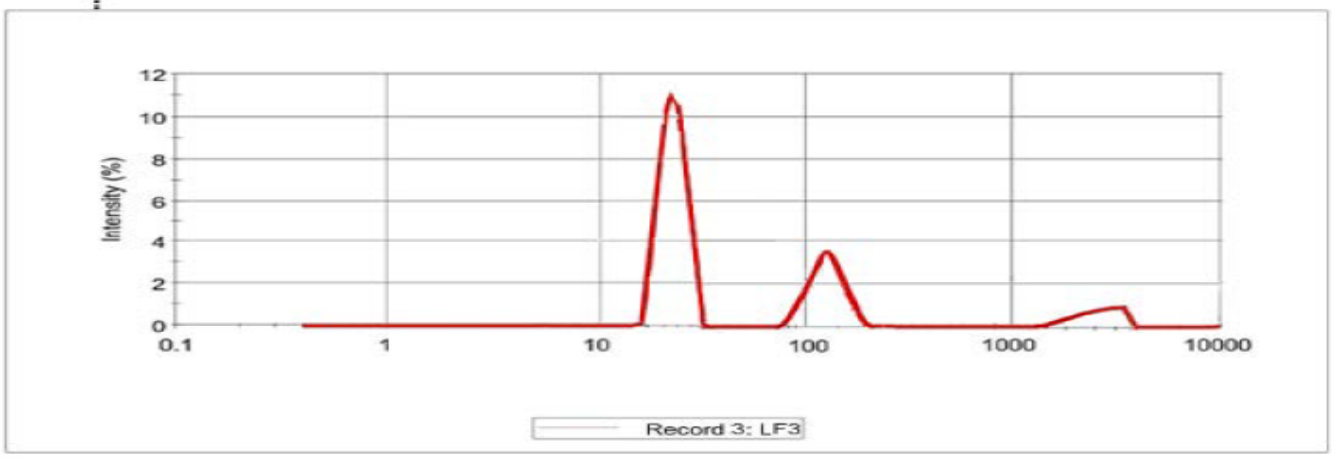


Fig. 18: Size distribution by intensity of F-2 formulation

The maximum number of Raloxifene Hydrochloride loaded Micellar dispersions is distributed in the range of 11.70 to 68.06 nm. The average particle size of Raloxifene Hydrochloride loaded Micellar dispersions is 25.37 nm and PDI was found to be 0.513

MALVERN PARTICLE SIZE ANALYSIS OF F-2

Size Statistics Report by Volume

Malvern

Sample Details

Sample Name: LF2
File Name: PM2.dts
SOP Name: mansettings.nano
Measurement Date and Time: Tuesday, Mar 14, 2017 5:20:43 PM

Z-Average (nm):	125.2 76 71	**Derived Count Rate (kcps):**	2424.1896543...
Standard Deviation (nm):	0	**Standard Deviation (kc...**	0
%Std Deviation:	0	**%Std Deviation:**	0
Variance:	0	**Variance:**	0

Size d.nm	Mean Volume %	Std Dev Volume %	Size d.nm	Mean Volume %	Std Dev Volume %	Size d.nm	Mean Volume %	Std Dev Volume %	Size d.nm	Mean Volume %	Std Dev Volume %
0.4000	0.0		5.615	0.0		78.82	23.3		1106	0.0	
0.4632	0.0		6.503	0.0		91.28	21.7		1281	0.0	
0.5365	0.0		7.531	0.0		105.7	11.7		1484	0.0	
0.6213	0.0		8.721	0.0		122.4	6.2		1718	0.0	
0.7195	0.0		10.10	0.0		141.8	0.8		1990	0.0	
0.8332	0.0		11.70	0.0		164.2	0.5		2305	0.0	
0.9649	0.0		13.54	0.0		190.1	0.4		2669	0.0	
1.117	0.0		15.69	0.0		220.2	0.4		3091	0.0	
1.294	0.0		18.17	0.0		255.0	0.0		3580	0.0	
1.499	0.0		21.04	0.0		295.3	0.0		4145	0.0	
1.736	0.0		24.36	0.0		342.0	0.0		4801	0.0	
2.010	0.0		28.21	0.0		396.1	0.0		5560	0.0	
2.328	0.0		32.67	0.0		458.7	0.0		6439	0.0	
2.696	0.0		37.84	0.0		531.2	0.0		7456	0.0	
3.122	0.0		43.82	0.5		615.1	0.0		8635	0.0	
3.615	0.0		50.75	4.3		712.4	0.0		1.000e4	0.0	
4.187	0.0		58.77	10.0		825.0	0.0				
4.849	0.0		68.06	20.6		955.4	0.0				

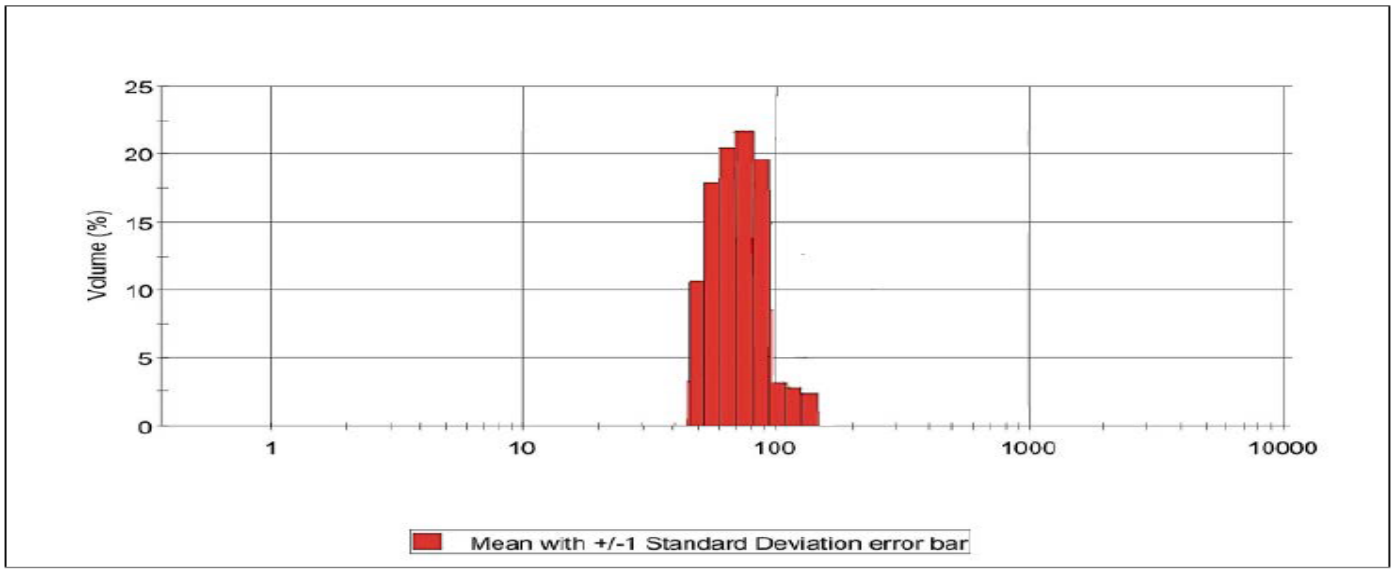


Fig. 19: Statistics graph of F-4 formulation

Size Distribution Report by Intensity

v2.1

Malvern

Sample Details

Sample Name:	LF2		
SOP Name:	mansettings.nano		
General Notes:			
File Name:	PM2.dts	**Dispersant Name:**	Water
Record Number:	2	**Dispersant RI:**	1.330
Material RI:	1.35	**Viscosity (cP):**	0.8872
Material Absorbtion:	0.10	**Measurement Date and Time:**	Tuesday, Mar 14, 2017 5:2...

System

Temperature (°C):	25.0	**Duration Used (s):**	80
Count Rate (kcps):	298.2	**Measurement Position (mm):**	3.25
Cell Description:	Disposable sizing cuvette	**Attenuator:**	6

Results

			Size (d.n...	% Intensity	Width (d.n...
Z-Average (d.nm):	125.27	**Peak 1:**	80.98	79.85	45.49
PdI:	0.679	**Peak 2:**	110.4	20.15	443.9
Intercept:	0.937	**Peak 3:**	0.000	0.0	0.000
Result quality	Good				

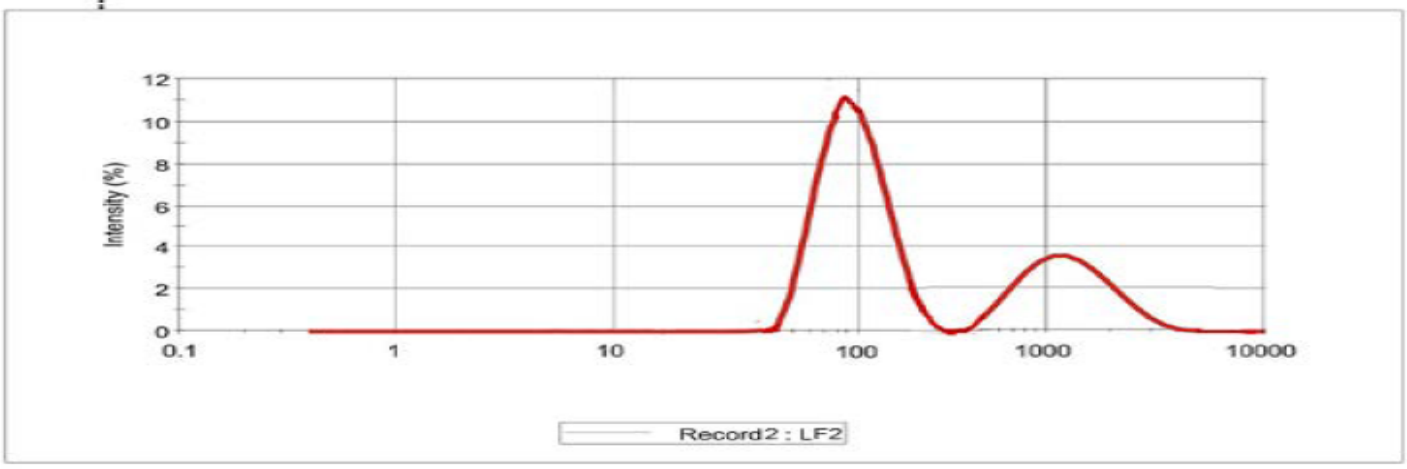


Fig. 20: Size distribution by intensity of F-4 formulation

The maximum number of Raloxifene Hydrochloride loaded Micellar dispersions is distributed in the range of 43.82 to 220.2 nm. The average particle size of Raloxifene Hydrochloride loaded Micellar dispersions is 125.27 nm and PDI was found to be 0.679

MALVERN PARTICLE SIZE ANALYSIS OF FORMULATION F-6

Size Statistics Report by Volume

Malvern

Sample Details

Sample Name: LF1
File Name: PM1.dts
SOP Name: mansettings.nano
Measurement Date and Time: Tuesday, Mar 14, 2017 5:35:14 PM

Z-Average (nm):	13.85124	**Derived Count Rate (kcps):**	3211.1422838...
Standard Deviation (nm):	0	**Standard Deviation (kc...**	0
%Std Deviation:	0	**%Std Deviation:**	0
Variance:	0	**Variance:**	0

Size d.nm	Mean Volume %	Std Dev Volume %	Size d.nm	Mean Volume %	Std Dev Volume %	Size d.nm	Mean Volume %	Std Dev Volume %	Size d.nm	Mean Volume %	Std Dev Volume %
0.4000	0.0		5.615	17.8		78.82	0.0		1106	0.0	
0.4632	0.0		6.503	20.1		91.28	0.0		1281	0.0	
0.5365	0.0		7.531	17.7		105.7	0.0		1484	0.0	
0.6213	0.0		8.721	13.2		122.4	0.0		1718	0.0	
0.7195	0.0		10.10	8.5		141.8	0.0		1990	0.0	
0.8332	0.0		11.70	4.8		164.2	0.0		2305	0.0	
0.9649	0.0		13.54	2.4		190.1	0.0		2669	0.0	
1.117	0.0		15.69	1.0		220.2	0.0		3091	0.0	
1.294	0.0		18.17	0.3		255.0	0.0		3580	0.0	
1.499	0.0		21.04	0.1		295.3	0.0		4145	0.0	
1.736	0.0		24.36	0.0		342.0	0.0		4801	0.0	
2.010	0.0		28.21	0.0		396.1	0.0		5560	0.0	
2.328	0.0		32.67	0.0		458.7	0.0		6439	0.0	
2.696	0.0		37.84	0.0		531.2	0.0		7456	0.0	
3.122	0.0		43.82	0.0		615.1	0.0		8635	0.0	
3.615	0.2		50.75	0.0		712.4	0.0		1.000e4	0.0	
4.187	3.2		58.77	0.0		825.0	0.0				
4.849	10.6		68.06	0.0		955.4	0.0				

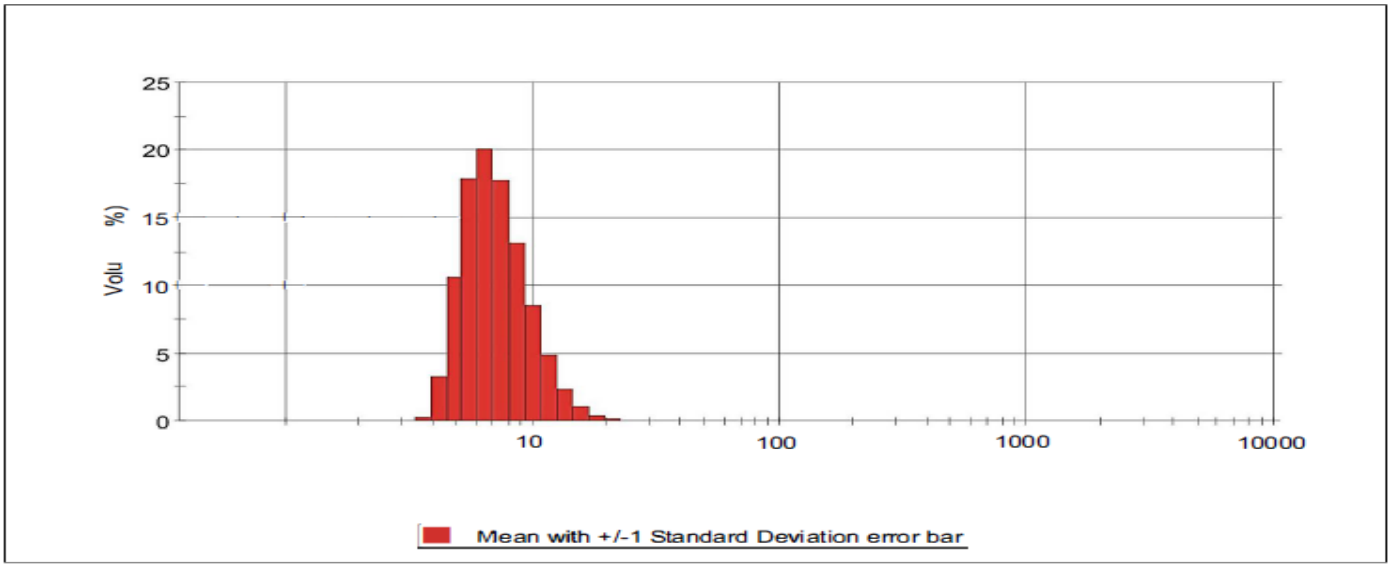


Fig. 21: Statistics graph of F-6 formulation

Size Distribution Report by Intensity

v2.1

Malvern

Sample Details

Sample Name:	LF1
SOP Name:	mansettings.nano
General Notes:	

File Name:	PM1.dts	**Dispersant Name:**	Water
Record Number:	1	**Dispersant RI:**	1.330
Material RI:	1.35	**Viscosity (cP):**	0.8872
Material Absorbtion:	0.10	**Measurement Date and Time:**	Tuesday, Mar 14, 2017 4:4...

System

Temperature (°C):	25.0	**Duration Used (s):**	60
Count Rate (kcps):	356.4	**Measurement Position (mm):**	4.65
Cell Description:	Disposable sizing cuvette	**Attenuator:**	9

Results

			Size (d.n...	% Intensity	Width (d.n...
Z-Average (d.nm):	13.85	**Peak 1:**	9.847	66.2	3.268
PdI:	0.465	**Peak 2:**	132.2	29.3	61.04
Intercept:	0.937	**Peak 3:**	4152	4.5	1036

Result quality : Good

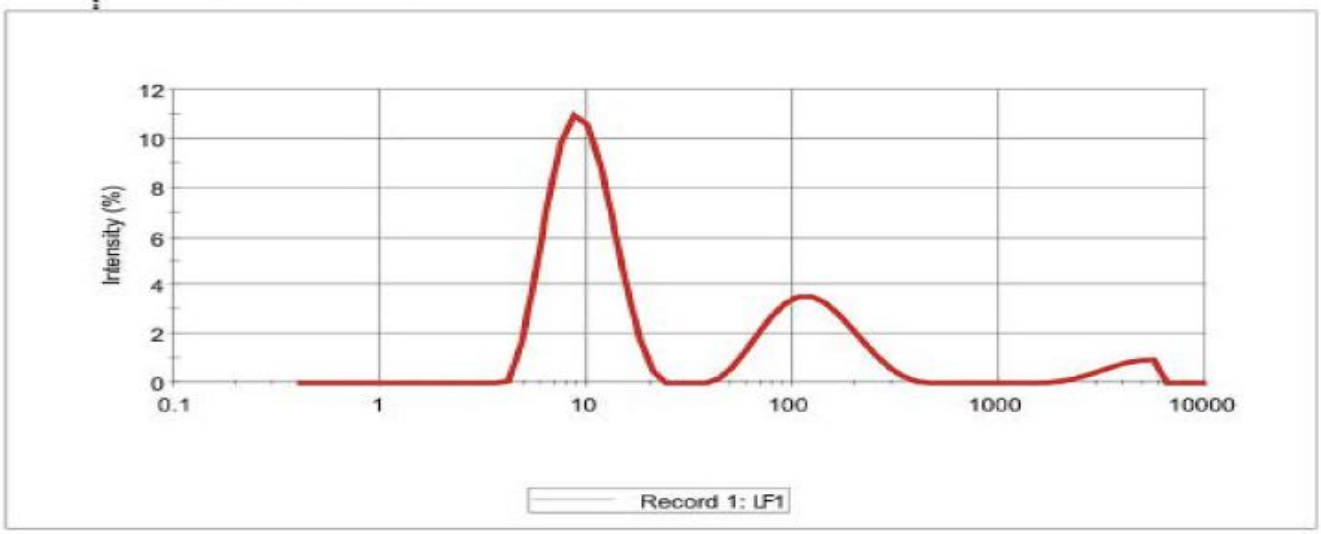


Fig. 22: Size distribution by intensity of F-6 formulation

The maximum number of Raloxifene Hydrochloride loaded Micellar dispersions is distributed in the range of 3.615 to 21.04 nm. The average particle size of Raloxifene Hydrochloride loaded Micellar dispersions is 13.85 nm and PDI was found to be 0.465

The average size of Raloxifene Hydrochloride loaded mixed micelles was found to be 25.37, 125.27 and 13.85 nm for F-2, F-4, and F-6 respectively. The F-4 prepared with using 500mg of L121 and 250mg of F127 had larger size which may be due to lamellar aggregates formed by long propylene oxide chains(PO) and short EO chains in L121 (Kung T. Oh et al., 2004). A 1:1 ratio of L121/F127 mixtures with total PBC concentraton of 1g shows small particle size. In case of PDI, the values are found to be 0.573, 0.679 and 0.465 for F-2, F-4 and F-6 respectively. So, it can be interpreted that F-6 micellar formulation will be more preferred for intestinal internalization due to it s comparatively smaller size and micelles shows best operated distribution algorithms (The Royal Society of Chemistry, 2015).

SOLUBILITY:

Solubility of the drug in PBS pH 6.8 is 0.2335 mg/ml and for optimized formulation, it was found to be 1.3003 mg/ml.

The optimized formulation shows 5.5 fold increase in solubility when compared to drug alone.

MALVERN ZETA POTENTIAL ANALYSIS OF OPTIMIZED FORMULATION (F-6)

Zeta Potential Report

v2.2

Malvern

Sample Details

Sample Name:	Zameela
SOP Name:	mansettings.nano
General Notes:	

File Name:	Zameela.dts	**Dispersant Name:**	Water
Record Number:	987	**Dispersant RI:**	1.330
Date and Time:	Wednesday, Mar 22, 2017 8:10:5...	**Viscosity (cP):**	0.8872
		Dispersant Dielectric Constant:	78.5

System

Temperature (°C):	25.0	**Zeta Runs:**	12
Count Rate (kcps):	251.1	**Measurement Position (mm):**	4.50
Cell Description:	Zeta dip cell	**Attenuator:**	6

Results

			Mean (mV)	Area (%)	Width (mV)
Zeta Potential (mV):	-59.37	**Peak 1:**	-59.37	100.0	10.7
Zeta Deviation (mV):	10.7	**Peak 2:**	0.00	0.0	0.00
Conductivity (mS/cm):	4.20	**Peak 3:**	0.00	0.0	0.00
Result quality :	Good				

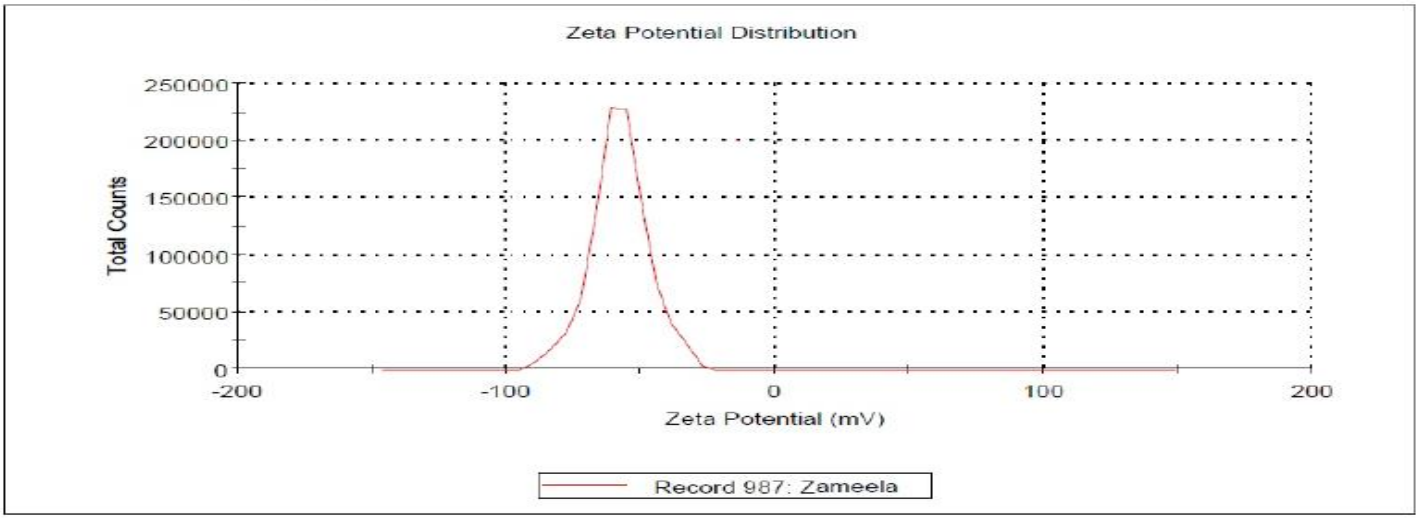


Fig. 23: Zeta Potential report of optimized formulation

The Zeta potential of the optimized formulation (F-6) was found to be -59.37 mV. This is due to highest HLB value of Pluronic F127, according to Anushree seth and Dhirendro S. Kothi (2012) the increased association of water (negatively charged hydroxyl groups) with the longer EO chain length of pluronic F127 resulted in increased negative zeta potential. Thus, the results obtained confirms the distribution of PEO unit by pluronic F127 on the surface of the micelles, and as said by Diego Chiapetta and Alejandro Sosnik (2007) the micelles with blocks made of PEO are sterically stabilized and undergo less opsonization and uptake by macrophages of RES. The higher negative charge shows high colloidal stability.(Anushree Seth & Dhirendro Kothi, 2012)

SUPER RESOLUTION AND CONFOCAL MICROSCOPY

Super resolution and confocal microscopy images were seen for the optimized micelle dispersion. The results are shown in the following figure 23 & 24.

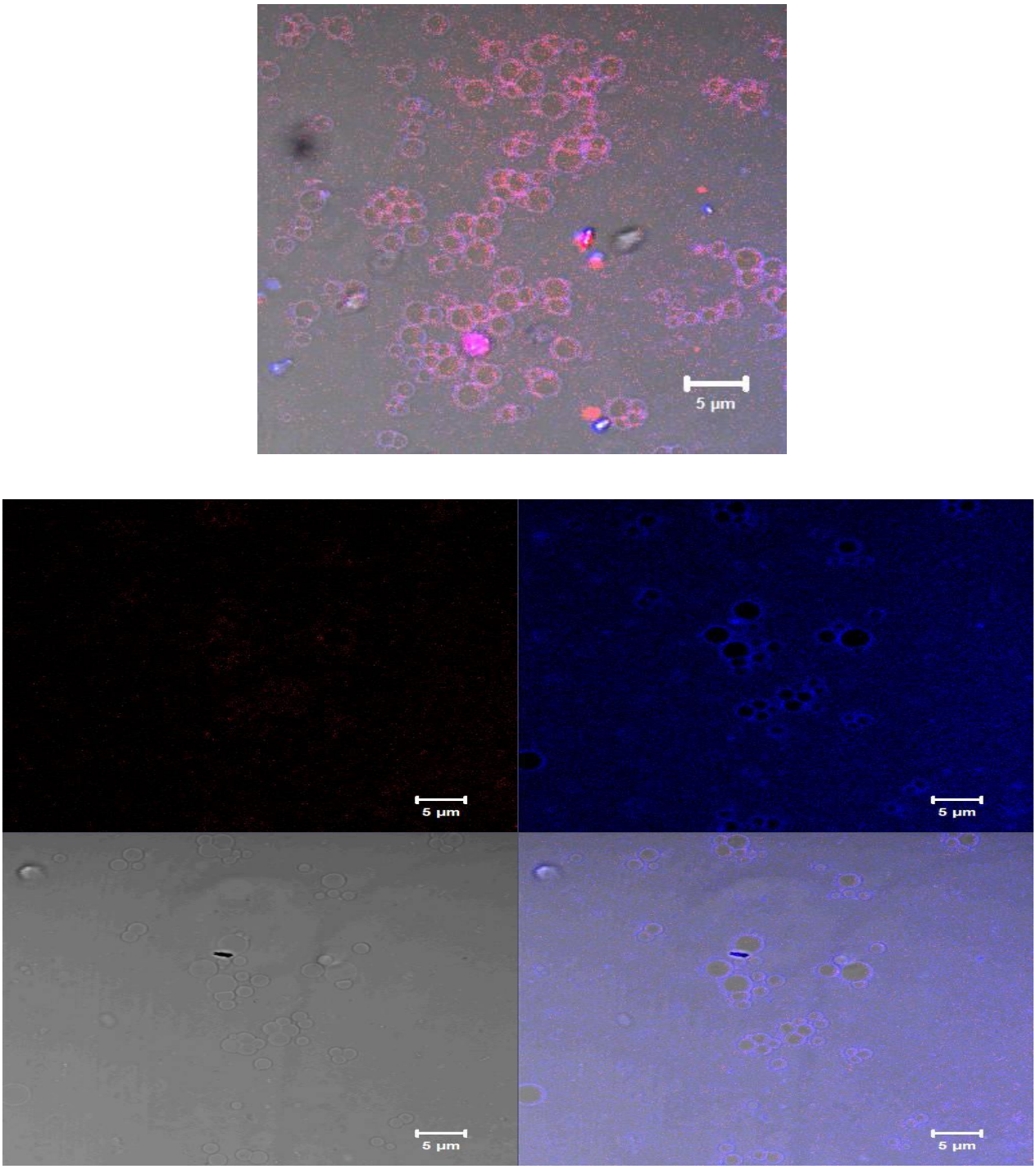


Fig. 23: 2D (2 DIMENSION) SUPER RESOLUTION AND CONFOCAL MICROSCOPIC IMAGES OF RALOXIFENE HYDROCHLORIDE MICELLE DISPERSIONS

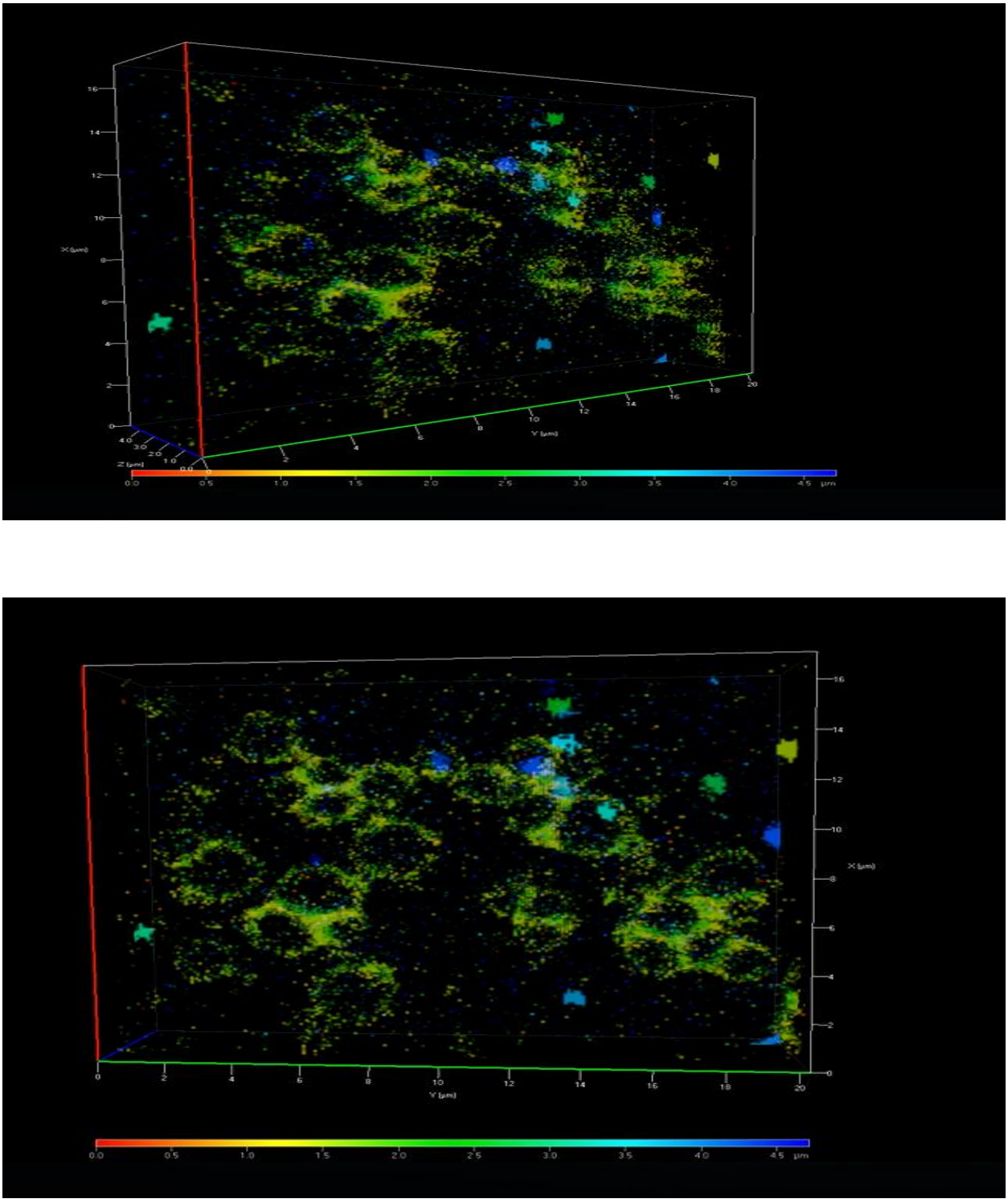


Fig. 24: 3D (3 DIMENSION) SUPER RESOLUTION AND CONFOCAL MICROSCOPIC IMAGES OF RALOXIFENE HYDROCHLORIDE MICELLE DISPERSIONS

The Super resolution and confocal microscopy analysis shows that the optimized polymeric micellar formulation F-6 is discrete and spherical in shape.

The Present investigation was undertaken with the objective to improve the poor aqueous solubility and the therapeutic efficacy of Raloxifene Hydrochloride by protection from extensive first pass metabolism and thereby improving the biovailability.

The aim of the present study is to formulate Raloxifene hydrochloride Polymeric Micelle drug delivery system using mixture of triblock copolymers, Pluronic L121 and F127 by thin film hydration method.

All the formulations were prepared, evaluated and the following conclusions are made

The possibility of Drug-Excipient interaction was investigated by FT-IR study. The FT-IR spectral analysis showed that there was no appearance or disappearance of any characteristics peaks of pure drug in the physical mixture of drug and polymer and in the optimized formulation, which confirms the absence of chemical interaction between drug and polymer.

The entrapment efficiency of all the formulations was between 92 to 96%. It was found to be 94.75, 95, 94.16, 92.5, 95.5 and 95.83% for F-1, F-2, F-3, F-4, F-5 and F-6 respectively.

The *in-vitro release* shows that the increase in concentration of hydrophilic polymer (pluronic F127) results in faster release of drug and increase in the hydrophobic polymer (pluronic L121) concentration, sustains the release rate of drug

Though increase in pluronic L121 concentration shows sustained release, the use of F127 is essential because it has longer PEO units. Thus using the same ratio of both L121 and F127 may result in prolonged circulation as micelles with blocks made of poly (ethylene oxide) sterically stabilized (stealth) and may undergo less opsonization and uptake by the macrophages of RES, allowing the micelles to circulate longer in blood.

Investigation of order and mechanism of drug release by plotting the *in-vitro* data of optimized formulation F-6 for zero and first order, higuchi, hixon crowell and Korsemeyer peppas equation were made. The result shows that the formulation F-6 follows zero order kinetics in which the regression was 0.9423. The ‘n’ value of Korsemeyer – Peppas equation was found to be 0.6476. From this it is concluded that the drug release follows non fickian diffusion.

The Malvern particle size analysis of formulations F-2, F-4 and F-6 was made and the average size of micelles was found to be 25.37, 125.27 and 13.85 nm respectively.

The Polydispersity Index of F-2, F-4 and F-6 was 0.573, 0.679 and 0.465 respectively.

From the result of particle size and polydispersity index it can be interpreted that F-6 micellar formulation will be more preferred for intestinal internalization due to its comparatively smaller size and micelles shows best operated distribution algorithms.

Solubility of the candidate drug in phosphate buffered saline pH 6.8 is 233.5 µg/ml and for optimized formulation F-6, it is found to be 1300.3µg/ml. Thus, it can be concluded that the optimized formulation shows 5.5 fold increase in solubility when compared to drug alone.

The Zeta potential of the optimized formulation was found to be -59.37 mV. The results obtained confirms the distribution of PEO unit by pluronic F127 on the surface of the micelles. Hence, the micelles are sterically stabilized and undergo less opsonization and uptake by macrophages of RES. The higher negative charge shows high colloidal stability.

The morphological studies using Super resolution and Confocal microscopy shows that the optimized formulation F-6 is discrete and spherical in shape.

Finally, the optimized formulation F-6 is concentrated by lyophilization using cryoprotectant (2.5%$^{w}/_{w}$ Sucrose).

FUTURE PLAN:

- To study the pharmacokinetic parameters of the candidate drug which may include area under the plasma concentration-time curve (AUC), elimination half life ($t^{1}/_{2}$), volume of distribution (V_d), mean residence time (MRT) and clearance (Cl).
- To perform the stability study
- To assess the improved *in-vitro* therapeutic efficacy.
- *in-vivo* studies and *in vitro-vivo* correlation studies.

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